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THE QUESTION OF NUCLEAR FUSIONS IN THE BLACKBERRY RUST, CAEOMA NITENS[1]

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INTRODUCTION

Strains of such obligate parasites as the mildews, smuts, and rusts, which are particularly pathogenic only when sowed on certain varieties of a host species, are now recognized as specialized or biologic forms. If the same condition obtains in case of the blackberry orange rust the conflicting reports coming in from observers in different regions would in part be understood. The writers have in culture several strains of this Rubus rust which can be readily distinguished. Moreover, the rust as it exists in certain plants is of such a nature as to suggest either that some strains are unstable or that the mycelia of two distinct strains may intermingle in the same sorus primordium (*5*).[2] Should the former be true, one is forced to explain how such an unstable strain could arise. If the latter be true, then a situation more conducive to the breeding of a hybrid rust as suggested in the paper referred to would be difficult to imagine. What the result of such a hybrid union would be depends first of all upon what becomes of the two nuclei which are brought together in a spore in consequence of the cell fusions. Should the nuclei fuse, as has been claimed, reduction would follow, and one might look for the effects of the crossing in the next generation. If, however, no nuclear fusion takes place, what then is the nature of the four sporidia? In prosecuting further studies, therefore, on the susceptibility of blackberry varieties to races of the orange rust, it has been found desirable to know exactly what the nuclei do during spore formation and germination. The life histories of the Endophyllum type of rusts and that of the short-cycled or *Caeoma nitens* rusts of blackberry are very similar. A knowledge of nuclear behavior in one group should lead to a clearer understanding of what takes place in the other group. A more detailed cytological investigation of the aeciospores of the Rubus rust during their development and germination has been made recently. The results of this study are reported at this time.

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[2] Reference is made by number (italic) to "Literature cited," p. 1024.

REVIEW OF LITERATURE

The Moreaus, in a series of papers, the last of which appeared in 1919 (*18*), have referred to previous work on the Endophyllum rusts, but it may be well to refer briefly to the conclusions reached by those who have studied nuclear behavior in the spores of rusts, especially forms the aeciospores of which produce promycelia.

Sappin-Trouffy (*27*) was the first to follow nuclear behavior in an aeciospore which in germinating produces a promycelium. He showed that in *Endophyllum euphorbiae-silvaticae* D. C. the mature spore contains two nuclei which, as the spore germinates, migrate, one following the other into the germ tube. They come together side by side as they divide, the two spindles lying parallel in telophase. After the four daughter nuclei have become distributed along the promycelium, two in one end and two in the other, three cross walls are laid down, cutting off one nucleus in each cell. It is all the more remarkable that Sappin-Trouffy should have come to the conclusion that no nuclear fusions occur in this form, since he had found nuclear fusions in every other rust, the germination of the teliospores of which he had studied critically. In Gymnosporangium, Melampsora, Thecopsora, and Cronartium, the fusion nucleus passes out into the promycelium and divides once, the two daughter nuclei moving apart and becoming separated by a cross wall. The two nuclei, though so separated, divide simultaneously and two more cross walls are laid down, completing the four-celled promycelium.

Maire (*14*) undertook a further cytological study of the Endophyllums. He says that in *E. sempervivi* the mature spores contain two nuclei, each with a large nucleole. The nuclei pass into the germ tube, where they divide conjugately (synergiquement), giving the four which are distributed along the promycelium and become separated by cross walls. He notes that *E. sempervivi* "de Gap" has spores that produce long germ tubes; he calls this form var. *aecidioides*, and suggests that it may belong to a long-cycled heteroecious rust. Maire points out that this variety has a great many more spermogonia than the typical *Endophyllum sempervivi* has, and recalls that de Bary had a form of this species of rust on an isolated plant which produced no spermogonia though normal aecidia were developed in abundance.

The situation is somewhat different in *Endophyllum valerianae tuberosae*. The young aeciospore, Maire says, has two nuclei when it is first developed, but one nucleus degenerates so that the mature spore contains only a single nucleus. When the spore germinates this nucleus passes out into the germ tube and is cut off below by a cross wall. Nuclear division follows, and a second cross wall is laid down. A sporidium is formed from the terminal cell only, since the other daughter nucleus degenerates. No spermogonia of this species were ever found by Maire.

Blackman (*3*) followed in some detail the stages in nuclear division in the promycelium from the teliospore of two species of Gymnosporangium. Unfortunately for the writers' particular purpose at this time, he failed to mention when the first cross wall was laid down. His Figure 38 of the two-nucleate promycelium is, in this respect, incomplete.

A form of *Endophyllum euphorbiae* whose aeciospores arise without cell fusions and have only one nucleus from the beginning,

was reported by Moreau (*20*). Other notes on this uninucleate form were published in two later papers (*21*, *22*). In a joint paper published with Moreau (*18*) additional information regarding this form is given, and the germination of the spores is described in greater detail. A few stages in the development of the promycelium are figured. The germ tube first contains a single nucleus; this nucleus divides, and later two nuclei separated by a cross wall are seen. The two nuclei divide so that there is nothing in the nuclear behavior in this *uninucleatum* form that differs from the course followed by the fusion nucleus when a teliospore containing a fusion nucleus germinates.

Hoffmann (*9*) was the first to carry on extensive cytological research on *Endophyllum sempervivi*. He had an abundance of material in culture, and apparently exercised great care in studying the details of spore formation and spore development. His best results were obtained by fixing aecia in Juel's solution and staining the preparations with Haidenhain's iron haematoxylin. Since the spores often germinate in the aecium under moist conditions, germination stages were studied from sections only 5 μ thick. Hoffmann says that the nuclei fuse soon after the binucleate spores are dislodged from the chains. This statement is supported by a number of excellent figures showing spores with a sculptured exospore and each spore with a single very large nucleus. Some of the nuclei are in the prophase, others are in metaphase, stages of division. The fusion nucleus does not always wait for germination to take place before it begins to divide. The nucleus usually completes its division before it migrates into the germ tube. One case is shown where the heterotypic division is occurring as the nucleus is about to pass through the germ pore. If a mature spore contains two nuclei they will not have nucleoles, and Hoffmann states that this is further proof that the heterotypic division has already occurred. On this basis he concludes that Sappin-Trouffy probably was mistaken in claiming that there are no nuclear fusions in *Endophyllum euphorbiae-silvaticae*. The second division may also take place occasionally before a spore germinates. Only one figure of a promycelium which affords evidence of a previous nuclear fusion is given in this paper. This figure shows a two-celled promycelium with a nucleus in each cell.

Kunkel followed his preliminary paper (*10*), showing that there is in America a short-cycled form of the blackberry orange rust *Gymnoconia interstitialis*, with a second paper (*11*) on nuclear behavior in the promycelia of this form. He says that the two nuclei in the aeciospore of the short-cycled form fuse so that the mature spore as it is about to germinate will contain only a single nucleus:

> A study of the binucleate spores during germination shows that they become uninucleate previous to the production of promycelia. Preparations made from cultures that were 15 hours old show a large number of uninucleate spores. In some of these preparations more than 90 per cent of the spores contain only one nucleus, thus demonstrating that during the early stages of germination the binucleate spores become uninucleate.

He figures two of these uninucleate spores, one of which has developed a germ tube of some length. He also shows a binucleated spore with a germ tube about the same length, suggesting that in this case the first division has taken place in the spore. The first

division of the fusion nucleus is figured in the diaster stage in the middle of a long germ tube. Although Kunkel saw spores with three or four nuclei, he found no evidence of disintegration of nuclei. He reports promycelia with more than four cells (excluding the stalk), but says that never were more than four sporidia developed in such cases. Kunkel agrees with Hoffmann that Sappin-Trouffy was probably wrong when he reported that there were no nuclear fusions in *Endophyllum euphorbiae-silvaticae*.

Poirault (*25*) described a new species, *Endophyllum centranthi-rubri*, on the red Valerian. The second paper by him (*26*) contains a very interesting summary showing the differences between *E. valerianae-tuberosae* and *E. centranthi-rubri*. Maire (*15*) had questioned whether there was really any difference between the two species. Poirault says he has seen both hosts in the same region, the red Valerian bearing rust but *Valeriana tuberosa* not. Accepting Maire's account of nuclear behavior in *E. valerianae-tuberosae*, Poirault concludes that there are two distinct rusts on these closely related hosts. It is in contrasting the two rusts that he states that the aeciospore of *E. centranthi-rubri* has only one nucleus from the time of its origin. When the mature uninucleate spore germinates, a two-celled promycelium is formed, a single sporidium developing from each cell. Exceptionally three sporidia develop from a promycelium. Poirault discovered in his later investigations that spermogonia were developed on plants which he had infected by sowing spores. He had not previously seen these structures on any of his other collections of this species.

The final paper by the Moreaus (*18*) gives in greater detail the results of their studies reported in preliminary papers (*22*, *16*, *17*) on *Endophyllum euphorbiae-silvaticae* and *E. sempervivi*. The rust on leaves was fixed in picro-formol, and sections 5 microns thick were stained with Haidenhain's haematoxylin. They found that the nuclei fuse soon after the spores are discharged from the chain and have begun to develop an exospore. If a spore develops four or six nuclei as the result of nuclear division, the supernumerary nuclei degenerate and only two are left to fuse. The spores were germinated on water, floated on glass slides where they were fixed and attached to the slide with collodion, and stained as before. The fusion nucleus undergoes a division in the germ tube, and the daughter nuclei separate and are cut off, one in each cell, by a septum. After the daughter nuclei have rounded up they contain no nucleoles. A second division occurs giving the ordinary four-celled promycelium. The Moreaus agree with Hoffmann as to nuclear fusion in this species, but they seem to differ widely with him in respect to certain important details.

According to Kursanov (*12*), forms of *Aecidium leucospermum* (*Ochrovsora Sorbi* Diet.) in which the aecia contain a large percentage of uninucleate spores, arising without cell fusions, are not rare in the vicinity of Moscow. The spores germinate with the regular type of long germ tubes, each having a single nucleus. Just why he was able to infect neither Sorbus nor Anemone with spores of this form is not clear, because, according to him, all aecia of this form contain some binucleate spores.

Two distinct strains of *Caeoma nitens* which are strikingly unlike the short-cycled form reported by Kunkel (*10*, *11*) were described

by the senior writer (*5*). It was shown that in one strain no spermogonia are developed, and that most of the aeciospores arise without cell fusions. These spores are uninucleate from their origin, and are very much smaller than are those with more than one nucleus. The uninucleate spores germinate readily, forming a two-celled promycelium, each cell eventually producing only one sporidium. The second strain is very similar to the first, except that it develops spermogonia. Chains of binucleate spores also occur in aecia of both strains. In some cases the basal cell of such chains is binucleate, but certain spores have two nuclei as a result of the division of its original nucleus. The assertion that the two nuclei in binucleate aeciospores of *Caeoma nitens* fuse before the spore germinates is questioned.

Lindfors (*13*) describes an interesting form of *Puccinia arenariae*, in which, he says, the haploid stage has been entirely suppressed. Every cell of the mycelium and of the reproductive structures is binucleate. The fusion nucleus migrates into the promycelium and divides. The spindle elongates and the daughter nuclei are then cut off, one in each cell, by a median septum. The two nuclei then divide simultaneously, and after reconstruction each cell of the promycelium has two nuclei; no other septa are laid down. A single binucleate sporidium develops from each of the two cells. With the germination of the sporidium and the infection of a new plant, the sporophytic condition is continued without a break. No cell fusions occur in the telial sorus, since the hyphal cells are already binucleate.

MATERIAL AND METHODS

Several horticultural varieties of blackberries (Rubus spp.) infected with the short-cycled or *Caeoma nitens* form of *Gymnoconia interstitialis* are grown in the greenhouse and in the field at Arlington Experiment Farm, Rosslyn, Va. Small pieces of leaves bearing aecia in various stages of maturity were killed in Flemming's fixatives. As an additional assurance that fully mature spores were embedded, the method described by Hoffmann (*9*) was also employed. Small sections of agar were cut out of the plate, and pieces of leaves bearing aecia containing large numbers of mature spores were laid in the holes. Melted agar was then poured over the specimens so as to cover the spores in agar. After the agar had solidified, pieces of it containing the specimens were fixed in the usual way. This material was sectioned from 5 to 10 μ thick and stained with the triple stain.

Two distinct methods were used in preparing the material for study of nuclear behavior. The first method of staining the spores was adapted to show their nuclei at maturity and just before germination begins. It consists in coating a No. 0 cover slip with a very thin layer of agar. After dusting the agar with spores, the slip is laid in a damp chamber, the time varying from one to several hours. The spores are then carefully flooded with one of Flemming's fixing fluids. After fixation the preparation is washed and run through the alcohols and stained with acid fuchsin and iodine green. Many of the spores are washed away, but there are always a large number left on the slip. These preparations show the nuclei so clearly that ordinarily the number present can be told with great certainty. Fixation of the cytoplasm and nuclei was very much better if the spores were

allowed to germinate before fixing. This method also proved satisfactory for a study of nuclear migration from the spore into the germ tube.

The second method was especially adapted for a study of germination stages. The spores are germinated on agar in Petri dishes in the usual way, and are allowed to fix in Fleming's fluid for several hours. In order to insure that no spores escape, melted agar may be poured over them, and after this agar has cooled the pieces are fixed as before. Sometimes the fixing agent is mixed with the melted agar, but this method is not fully satisfactory. Sections of this material were cut 20 μ thick and parallel to the surface of the agar. Stained with the triple stain such preparations are highly satisfactory, as they often show the spores with their entire germ tubes or promycelia. For the mature but ungerminated spores to be stained in toto the acid-fuchsin and iodine-green method is superior.

Having selected an infected plant from which material was to be obtained for a cytological study of aecia, series of germination studies were carried out in order that there be no mistake as to the nature of the strain of rust in that particular plant and in the leaf, pieces of which were fixed. Conversely, if a cytological study of mature spores or of promycelia was to be made, it was found very desirable to fix some of the aecia from which spores were obtained, so that a study of the spore chains could be made later.

GYMNOCONIA INTERSTITIALIS, FORM CAEOMA NITENS

The writers have in culture, as noted above, at least four distinct short-cycled strains of the Rubus rust, Gymnoconia. In two strains most of the aeciospores originate without cell fusions and are uninucleate; in the other two strains cell fusions regularly occur, so that most of the spores are binucleate when they are cut off as the spore initial divides. The uninucleate spores of the first two strains develop two-celled promycelia, the binucleate spores of the last two develop four-celled promycelia (*5*).

BINUCLEATE SPORES

The question to be determined first is whether or not the two nuclei originally contained in the spore which is to develop a four-celled promycelium fuse, either as the spore comes to maturity or during any stage of germination. In order to ascertain the facts in this case, the different points in the development and germination of the spores at which such fusions might be looked for have been kept in mind.

Naturally the cytologist looks first of all at the spore chains, following them spore by spore from the basal cells upward. The spores of the short-cycled orange rust are not shot out of the sorus as soon as mature, but tend to cling together and heap up and finally fall out in bunches. There would be an opportunity for nuclear fusions between the time the spores are separated from the chain and the time of their germination, which may even occur in the sorus, especially in moist weather. The longer the resting period of the fusion nucleus the more easily it should be detected. In case of a very short resting period the critical stage might be overlooked, especially if, as Hoffmann (*9*) states for *Endophyllum sempervivi*,

the fusion nucleus divides heterotypically as the spore reaches maturity; the old spores as well as the immature ones would be binucleate.

Fusion might be delayed until germination had actually begun. Two nuclei would be seen in the spore until just before they passed through the germ pore. This would necessitate a critical study of germ tubes which would show a single nucleus at some stage in their growth, if fusion had occurred. When a rust goes over from its long-cycled habit to the short-cycle type, one would expect that nuclear fusions, if they were to occur, would be delayed as long as possible. The last place where such a fusion could be effected would be immediately after the two nuclei had come to rest in the middle of the germ tube. Nuclear divisions would follow at once, so that again the fusion stage might escape notice. Failure to find fusion nuclei at any of the stages mentioned would be adequate proof that no fusions occur, if a detailed account were given of what the nuclei do during these periods. Abundant material was available for study, and no difficulty was encountered in following the course of nuclear behavior step by step from the time the two nuclei come together in the fusion cell until the four sporidia are cut off from the cells of the promycelium.

Examination of hundreds of slides of sections of aecia containing spores in every stage of development, from their origin until the spores are dislodged from the chains and are heaped up in the aecia, fails to disclose the slightest evidence of nuclear fusions up to this time. The paired nuclei of the fusion cell divide conjugately, and the spore initial is cut off. Again there is a conjugate division, after which the aeciospore is cut off with the interstitial cell below. The spore matures and rounds up, and as the exospore becomes distinguishable the two nuclei still remain apart and distinct.

It is the cases where only one nucleus shows in a spore section that compel one to use other methods for following the behavior of the nuclei, because it is often impossible to locate with certainty the same spore in the next serial section. Where spores are stained in toto so that the nuclei show clearly, no such objection can be raised. Preparations of spores stained in toto, after having been allowed to remain under conditions favorable for their coming to maturity and for their passing through the stages preliminary to germination, still show two distinct nuclei. (Fig. 1, pl. 1.) The germ tube begins to appear (fig. 2, pl. 1), and still the two nuclei, each with its nucleole, are clearly visible. As the tube elongates, the nuclei undergo a slight change of form, becoming pear-shaped or pointed, the beak pointing toward the germ pore. (Fig. 3, pl. 1.) The nucleoles, which are always very small in this species, are still visible at this stage, provided the nuclei are well stained. Thousands of spores in these stages stained in toto with acid fuchsin and iod ine green have also been studied without any evidence of nuclear fusion having been found. In sectioned preparations of germinating spores, the nuclei are more delicately stained, so that their nucleoles show much more clearly.

The migrating nuclei elongate still more as they enter the germ tube and if fixed at this stage the beaks usually stain heavily, but the pouchlike lower ends maintain their delicate structure. The

peculiar undulating, wavy or S-shaped forms of the drawn-out nuclei (figs. 5, 8, pl. 1) contribute to the orientation of the nuclei in the tube later. Just before the nuclei have completed their passage out of the spore and into the tube they begin to shorten, and the undulating forms which they took while passing through the pore are changed. The process by which two nuclei which migrate side by side through the pore become oriented in the tube, one beyond the other, can be readily understood. (Fig. 6, pl. 1.) As the nuclei pass out of the spore into the tube they appear to be preceded or surrounded above by a light region in the cytoplasm devoid of granular substance and capped by a mass of cytoplasm which stains readily (figs. 6, 8, pl. 1) as though the nuclei were forcing the tube contents outward instead of the nuclei being drawn out, as really may be the case. Either the beak end of one nucleus is slightly in advance of the other (fig. 6, pl. 1) or the outer ends of the elongated nuclei are curved in such a way that one nucleus in its contraction slips by or around and over the other (fig. 11, pl. 1). In shortening and thickening still further, both nuclei become more or less crescent-shaped or arched outward. (Figs. 10–13, pl. 1.) Such a stage, especially if it be poorly fixed, might easily be mistaken for a telophase stage of a heterotypic division.

Although the nuclei often migrate together (figs. 3, 4, 5, 8, pl. 1), or one follows somewhat behind the other (figs. 6, 7, 9, pl. 1), one nucleus may sometimes complete its passage and begin to round up in the tube before the other has entered the germ pore region (fig. 10, pl. 1). Having reached the middle of the elongated germ tube, the nuclei regain their even spherical contour, showing delicately staining chromatin and a distinct nucleole. (Fig. 14, pl. 2.) If the presence of a nucleole in each nucleus is any further proof that nuclear fusion has not yet occurred, we may be sure that no fusion has taken place up to this time, either in the spore or during migration in spore germination.

EXPLANATORY LEGEND FOR PLATE 1

FIG. 1.—Binucleate aeciospore soon after being freed from the chain.

FIG. 2.—Early stage in spore germination. The nuclei show large nucleoles deeply stained with acid fuchsin.

FIG. 3.—Later stage in spore germination. One nucleus elongated, directed toward the germ pore, the other showing nucleole distinctly. The length of the germ tube is about the diameter of the spore. Cytoplasm of the spore becoming vacuolate.

FIG. 4.—Slightly later stage. The beak of one nucleus entering the germ pore. Other nucleus somewhat elongated.

FIG. 5.—Nuclei showing chromatin in a reticulate condition, the beaks deeply stained. Nucleole visible in one nucleus.

FIG. 6.—Nuclear beaks entering the germ tube, and showing granular chromatin mass. Migrating nuclei surmounted by a more densely stained cap of cytoplasm, each nucleus showing a distinct nucleole.

FIG. 7.—Stage somewhat similar to the preceding, except that one nuclear beak overlies the other and is somewhat curved at the end.

FIG. 8.—Nuclei, proceeding together through the germ pore. They are drawn out into S-shaped figures, surrounded by a clear area in the cytoplasm of the germ tube. Cytoplasm above the nuclei more deeply stained.

FIG. 9.—Nuclei migrating from the spore, one in advance of the other. The outer one surrounded by a hyaline area, and more densely stained.

FIG. 10.—Later stage of another type of migration but similar to the preceding, one nucleus having reached the middle of the germ tube, the other, much elongated, passing through the germ pore.

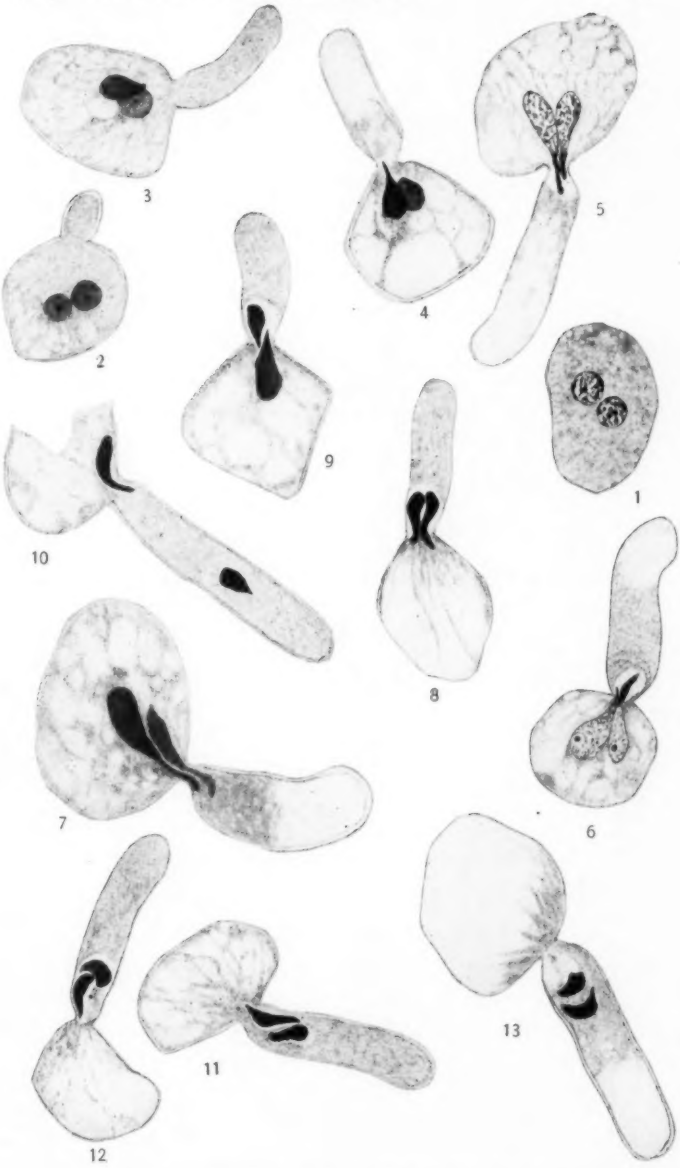
FIG. 11.—Shows how one of the two nuclei which pass through the germ pore together slips by the other and rounds up, the two coming to lie in the germ tube one beyond the other.

FIG. 12.—Might be interpreted as being somewhat earlier stage than figure 11. Shows how the elongated and curved nuclei slip by one another in becoming oriented in the germ tube.

FIG. 13.—Two nuclei completely oriented and beginning to round up. Both somewhat crescent shaped.

(All figures were drawn with the aid of a camera lucida, Zeiss 2 mm., 1.40 aper. and 1.5, 1.30 aper. apochromat lenses, and 8 comp. oc. Nos. 1, 5, and 6, are from material sectioned 20 μ thick and stained with Flemming's triple stain. Nos. 2, 3, 4, 7, 8, 9, 10, 11, 12, and 13, are from material stained in toto, with acid fuchsin and iodine green.)

(The exospore is not shown in any of the figures.)



Stages in nuclear migration in the germination of binucleate spores
(For explanatory legend see page 1010)

Stages in nuclear division of binucleate aeciospores
(For explanatory legend see page 1011)

The possibility that the nuclei might fuse as they lie in the middle of the promycelium has not been overlooked, although, to the writers' knowledge, no one has ever reported such a phenomenon in the rusts. This stage has been studied most critically without finding any suggestion that such a thing ever occurs here. The nuclei in this stage may be fixed so that their chromatin strands stain most delicately. Following the stage in which the chromatin appears finely reticulate (fig. 14, pl. 2), as is usually found in resting nuclei, the network becomes heavier, and more deeply-staining granules are distributed along the threads (fig. 15, pl. 2). A little later more evenly staining strands of chromatin of different lengths appear to be somewhat peripherally arranged in the nucleus, leaving much of the nuclear region clear and unstained. (Fig. 16, pl. 2.) In one case the chromatin strands were clearly polarized, and at least 14 loops were counted. (Fig. 42, pl. 4.) In the metaphase stage and very early anaphase stage the spindles are certainly very short. (Fig. 17, pl. 2.) The two nuclei may and do most often divide simultaneously, but they do not necessarily divide conjugately. The spindles in the metaphase may lie one ahead of the other (fig. 18, pl. 2), so that they are not exactly side by side, although they may become so in the telophases (fig. 20, pl. 2). One nucleus may complete its division while the other is still in the anaphase. (Fig. 17, pl. 2.) Figure 19, Plate 2, shows again one nucleus in a more advanced stage of division than the other, which is apparently in an anaphase stage, the chromatin moving along the spindle fibers. The upper nucleus shown in Figure 18 (pl. 2) is in a slightly later stage of division, when most of the chromosomes have reached the poles, but some are still lying along the spindle. A distinct cone of fibers is seen in the lower nucleus of this figure, again suggesting a short spindle comparable to that shown in Figure 30, Plate 3.

When the chromatin masses have reached the poles and begin to separate, they are still connected by a strand or strands of fibers. (Fig. 20, pl. 2.) It is soon after this stage that one spindle slips by the other, a pair of daughter nuclei going to each end of the promycelium. Figures 21, 22, and 23 (pl. 2) show successive stages of the nuclear distribution. No vestige of fibers or strands connecting sister nuclei is evident in Figure 23, where one pair of sister nuclei has completely slipped ahead of the other. A similar stage is shown

EXPLANATORY LEGEND FOR PLATE 2

FIG. 14.—The germ tube more elongated. The nuclei completely rounded up, and lying one beyond other, and not side by side. Nucleoles showing plainly, lying in a rather distinctly stained granular chromatin.

FIG. 15.—Chromatin masses showing clearly in early prophases of division.

FIG. 16.—Chromatin masses in bouquetlike arrangement of chromatin threads.

FIG. 17.—One nucleus has completed the division. The other shows delayed division, probably early anaphase.

FIG. 18.—One nucleus in early anaphase, the other in telophase; a number of distinct fibers extend between the two polar masses of chromatin.

FIG. 19.—Both nuclei undergoing division. One in telophase, with fibers still stretching between the polar masses, the other the chromatin bodies still scattered along the spindle.

FIG. 20.—The nuclei dividing, their spindles lying almost parallel in the promycelium.

FIG. 21.—Late stages in nuclear division, one spindle slightly ahead of the other.

FIG. 22.—Late stages in nuclear division, one spindle lying at an angle in the bent promycelium.

FIG. 23.—Nuclear division nearly completed. The spindles with their reorganizing pairs of nuclei have slipped by each other, and the four daughter nuclei will come to lie in a line along the promycelium.

FIG. 24.—The pairs of sister nuclei reorganized and separated in the promycelium by the median cross wall.

(All figures were drawn with the aid of a camera lucida, Zeiss 2 mm., 1.40 aper. and 1.5, 1.30 aper. apochromatic lenses, and 8 comp. oc. All drawings on this plate are from material sectioned 20 μ thick, and stained with Flemming's triple stain.)

(The exospore is not shown in any of the figures.)

in Figure 44 (pl. 4), where there is no doubt as to the relation of the nuclei. Ordinarily a septum is laid down as the nuclei of the pairs are becoming reorganized, so that one is certain that the two end cells possess sister nuclei. (Fig. 24, pl. 2.) The two sporidia formed at one end after the laying down of the cross walls will be the direct descendants by vegetative division of one of the two nuclei entering the fusion cell at the origin of the spore chain. The two sporidia at the other end will be alike, having exactly the same genetic constitution as the other nucleus which entered the fusion cell. There has been no nuclear fusion, no reduction division, and therefore no chance for a mixing and recombination of hereditary elements.

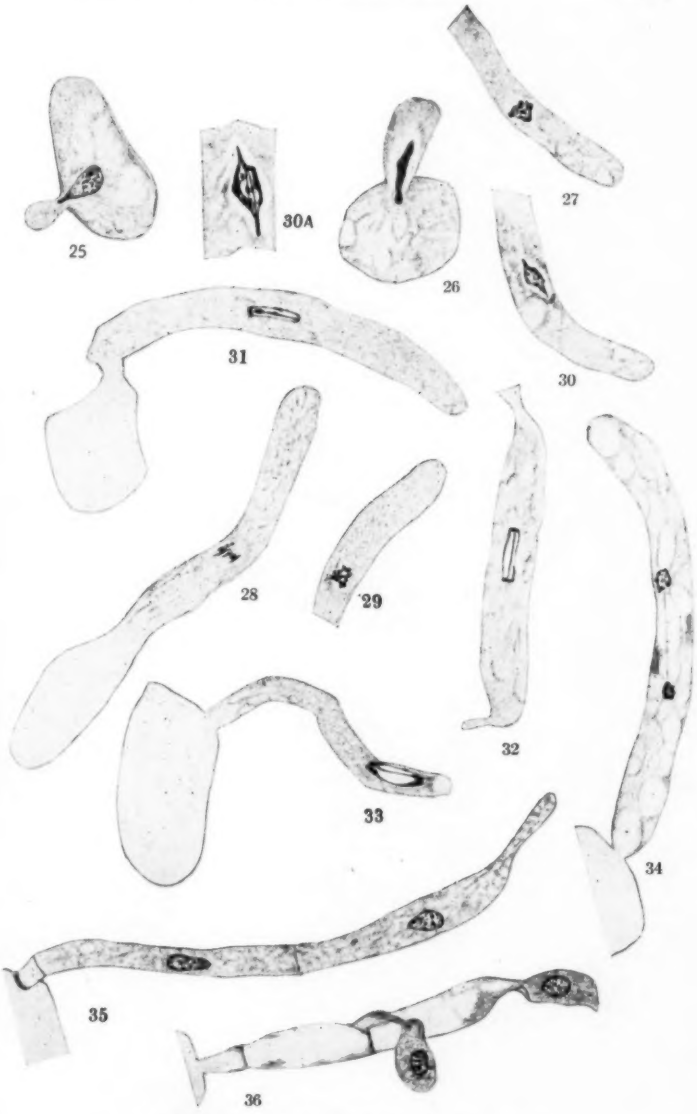
UNINUCLEATE SPORES

Having followed the binucleated spores from their origin up to and through their germination stages without finding any evidence of nuclear fusion, it is easy to understand what happens if for any reason a polynucleate or a uninucleate spore is developed. The germination of uninucleate aeciospores and the formation of two-celled promycelia by such spores was described in an earlier paper by Dodge (*5*), but the cytological details in the migration and division of the spore nucleus had not at that time been fully worked out. Recent studies of this form show that while a binucleate or polynucleate spore may arise by nuclear division in a chain, the basal cell of which has only one nucleus, the writers suspect that ordinarily after a spore has been dislodged from the chain no further division takes place in the spore. No cases of nuclear division have as yet been found within such spores as they are about to germinate or have actually begun the process. The single nucleus elongates into a beak (fig. 25, pl. 3), which passes through the germ pore with the pouchlike lower part following.

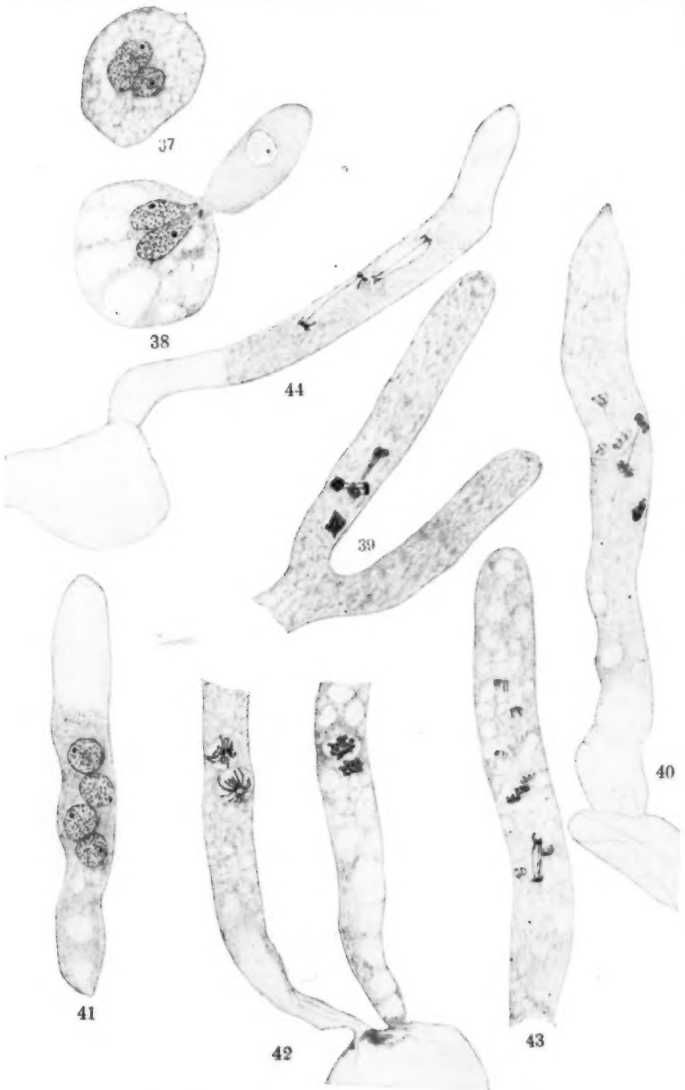
When well into the germ tube, the nucleus shortens and becomes crescent-shaped, meanwhile passing out into the middle of the tube where it will round up, still showing its nucleole. A septum may be laid down at this time, cutting off the outer end of the tube with the single nucleus. The single nucleus then passes through prophase stages of division (fig. 27, pl. 3) similar to that noted for each one of the pair of nuclei (fig. 15, pl. 2). In Figure 28, Plate 3, is shown what is apparently a lateral view of an equatorial plate arrangement of the chromosomes, while Figure 29, Plate 3, is probably a somewhat

EXPLANATORY LEGEND FOR PLATE 3

FIG. 25.—The single nucleus which is drawn out into a sharp beak shows a distinct nucleole.
FIG. 26.—Much elongated nucleus passing through the germ pore, surrounded by a hyaline area in the cytoplasm.
FIG. 27.—The nucleus has passed out to the middle of the promycelium, and is now in early stage of division similar to that shown in Figure 15 of the binucleate aeciospore.
FIG. 28.—Lateral view showing the chromosomes arranged along the equatorial plate.
FIG. 29.—Rather oblique view of chromosome masses in equatorial plate.
FIG. 30.—Later stage in nucleus division. Chromosome masses on a distinct spindle, which is more highly enlarged in Figure 30A.
FIG. 31.—Somewhat later stage. Some of the chromatin masses have already reached the poles.
FIG. 32.—The chromatin has gone to the poles, the spindle fibers still persistent.
FIG. 33.—Late telophase, distinct fibers stretching between the polar masses. End of germ tube cut off.
FIG. 34.—Daughter nuclei completely separated and reorganized. Long, delicate fibers still visible.
FIG. 35.—The sister nuclei reorganized and separated by a cross wall in the promycelium, the outer apical cell elongating in preparation for the formation of a sporidium.
FIG. 36.—Mature promycelium, showing two uninucleate sporidia.
(All figures were drawn with the aid of a camera lucida, Zeiss 2 mm., 1.40 aper. and 1.5, 1.30 aper. apochromatic lenses, and 8 comp. oc. All drawings on this plate are from material sectioned 20 μ thick and stained with Flemming's triple stain.)
(The exospore is not shown in any of the figures.)



Nuclear migration and division in uninucleate aeciospores
(For explanatory legend see page 1012)



Nuclear behavior during germination of multinucleate aeciospores
(For explanatory legend see page 1013)

oblique polar view. The chromosome bodies are so small and closely crowded that the number drawn is probably only an approximation of the number actually present. Figure 30A, Plate 3, which is the spindle of Figure 30 enlarged, shows a very definite spindle, though the chromosomes seem to be already moving toward the poles. It is possible that the stage showing a definite equatorial arrangement does not persist long, since it has been found so rarely, whereas telophase stages are very common. Nuclear division occurs, and the polar masses become separated (figs. 31–34, pl. 3) and show the connecting fibers. After reorganization the second septum is laid down (fig. 35, pl. 3) and the sporidia will be developed (fig. 36, pl. 3).

POLYNUCLEATE SPORES

Turning to the polynucleate spores (fig. 37, pl. 4), it will be found that the behavior of each of the nuclei is precisely the same as it would have been if the spore had been provided with only one or two nuclei instead of three or more. In one spore, for example, three nuclei were clearly seen migrating together; in another spore one nucleus had completely passed into the tube and had rounded up, while the other two were just entering the pore. (Fig. 38, pl. 4.) Having reached the middle of the promycelium, the three, four (fig. 41, pl. 4), or more nuclei round up, each showing a nucleole. Nuclear division then proceeds by the formation of spindles and the division of each nucleus into two daughter nuclei, as was found for the single nucleus of a uninucleate spore, or each of the nuclei of a binucleate spore.

The promycelium from a polynucleate spore is very likely to be deformed or abnormal. In some instances two separate germ tubes are formed from the same spore, each taking a pair of nuclei. (Fig. 42, pl. 4.) One of the two nuclei in the left tube distinctly shows 14 chromatin loops in a large clear nucleus, and is probably a prophase stage comparable to that of Figure 16, Plate 3. Figure 39, Plate 4, shows a branched promycelium with all three dividing nuclei still in one arm. Stages are readily found showing three (fig. 40, pl. 4), four, or five (fig. 43, pl. 4) spindles arranged at various angles to each other and lying about in the broad tube. It is clear that the

EXPLANATORY LEGEND FOR PLATE 4

FIG. 37.—A three-nucleate spore just beginning to develop a germ tube. Nucleoles showing clearly in the granular chromatin.

FIG. 38.—A later stage in the germination of a three-nucleate spore. Two nuclei elongated and showing beaks proceeding through the germ pore. The third nucleus, already in the tube, shows a distinct nucleole.

FIG. 39.—A branched promycelium, showing the three nuclei undergoing division. In the lower nucleus the chromosomes are still at the equatorial region of the spindle. The other two are in more advanced stages of division, their axes being at right angles to each other.

FIG. 40.—Three nuclei have already completed their division. Delicate fibers between two of the pairs are seen.

FIG. 41.—Four nuclei as they lie in the promycelium after they have migrated out of the spore. This stage comparable with that shown in Figure 14, Plate 2, of the binucleate aeciospore.

FIG. 42.—A rather unusual type of germination of a four-nucleate aeciospore, two nuclei showing in each of the separate germ tubes. The nuclei in the promycelium at the left show a boquetlike arrangement of the chromatin threads.

FIG. 43.—Five nuclei completing division. The two corresponding polar masses from each of the dividing nuclei can be recognized by careful focusing.

FIG. 44.—The two spindles of a division of a binucleate spore slipping by one another. This figure was obtained from material which regularly shows uninucleate aeciospores.

(All figures were drawn with the aid of a camera lucida, Zeiss 2 mm., 1.40 aper. and 1.5, 1.30 aper. apochromatic lenses, and 8 comp. oc. All drawings on this plate are from material sectioned 20 μ thick and stained with Flemming's triple stain.)

(The exospore is not shown in any of the figures.)

nuclei are not all in the same stages of division. In Figure 39, Plate 4, the chromosomes of the lowest nucleus are still arranged equatorially on the spindle, while those of the other two nuclei have nearly all reached the poles. Such cases of the delayed division of one nucleus in the promycelia of binucleate spores are shown in Figures 18 and 19, Plate 2. All evidence supports the view that each nucleus in a spore is capable of independent and individual action during migration and division. Binucleate spores are found occasionally in preparations from aecia containing mostly uninucleate spores. Figure 44, Plate 4, represents such a case. This figure furnishes further evidence of the manner in which one spindle slips by the other. (Figs. 20 to 23, pl. 2.)

The number of cells in a promycelium is largely determined by the number of nuclei originally present in the mature spore. The particular form of the promycelium, whether it be double, branched, or lobed, is not accounted for readily, but the number of cells and sporidia it will develop depends on the accidental distribution of the pairs of daughter nuclei. Dying of certain cells or degeneration of certain nuclei may occur, but if such things do not happen and all goes well there will be eventually twice as many sporidia on a promycelium as there were nuclei in the mature spore.

DISCUSSION

Evidence has been presented to prove that there are no nuclear fusions in the life of the short-cycled *Caeoma nitens* strains of the Rubus rust. It has been held constantly in mind that it would be necessary to follow the pair of nuclei step by step from the time they first come together in the fusion cell until they divide and become separated by a cross wall in the promycelium.

A nuclear fusion in the teliospore has come to be regarded as the one constant and most essential process in the life of every rust. It is now 30 years since Sappin-Trouffy (*27*) was so bold as to assert that there are no nuclear fusions in *Endophyllum euphorbiae-silvaticae*. Even though the Moreaus have proved beyond a doubt that Sappin-Trouffy was right, the significance of his discovery is yet to be appreciated. This part of his work is not mentioned even by either Kursanov or Lindfors. This attitude may be due to the fact that most students of the rusts have been more particularly interested in finding out about how the two nuclei get together in the basal cell, with the result that the phase of the question dealing with what becomes of them later has been somewhat neglected. What a different reaction was stimulated by Hoffmann's (*9*) announcement that the two nuclei in the aeciospores of *E. sempervivi* fuse as the spore comes to maturity. The statement is always referred to and accepted without question because it was what everyone expected. In his discussion suggesting the possibility that Endophyllum is a reduced form and not primitive, Fromme (*6*) says: "The assumption of the teleutosporic method of germination by an aecidiospore is not a difficult conception, since nothing more than the fusion of nuclei in the spore should be necessary for its accomplishment." *E. euphorbiae* and *Caeoma nitens* are clearly reduced forms, and they became such even without nuclear fusions.

After all the years of cytological work dealing with nuclear fusions, reduction divisions, pollen tetrad formation, and other phases of the cycles associated with fertilization in green plants, as well as fungi,

it is not easy to imagine that there could be developed normally by a rust a four-celled promycelium with four sporidia as a result of "sexual" fusions without there having been a reduction division. Yet that is what the discovery by Sappin-Trouffy means, and the discovery of the same course of development in *Caeoma nitens* means that there are probably other similar cases yet to be brought to light.

It is a fact exemplified many times over in the evolution of plants and animals that the factors that stand for or represent a morphological structure are not indissolubly connected with those factors that determine how the structure shall function. If an organ ceases to perform its original function and takes on a new function, or takes over work performed heretofore by another organ which has been suppressed in evolution, the scientist may give the organ a new name which will more nearly connote the change of function. He never forgets, however, what that organ represents in phylogeny, neither does he apply to it a name by which the suppressed organ is known. When a long-cycled rust like *Gymnoconia interstitialis* becomes short-cycled not only by suppressing the telial stage but also by omitting the most essential physiological activity consummated heretofore in the teliospore, the fact is not explained by referring to the case as teratological or as a monstrosity. Promycelia which develop seven or eight sporidia need no longer be called abnormal, because it is now known that these multicellular promycelia come from multinucleate spores in which no nuclear fusions occur. The factors representing septate germ tubes or promycelia and sporidia are active in *Caeoma nitens*. There occurs a pseudofertilization in the cell fusions without an ultimate nuclear fusion, and this is followed by a pseudoreduction by cell division in the promycelium.

According to all the evidence to date, the nuclei in the auxiliary cells of the Florideae take no part by way of nuclear fusions in the inauguration of spore production, yet these same auxiliary cell fusions play the leading rôle, or are the first essentials, in the development of carpospores in all but the simplest of the red algae. The nuclei in the ooblastema filaments are diploid in consequence of a previous sexual union between sperm and egg. A fusion between a diploid and a haploid nucleus would not be expected in connection with the fusions between auxiliary and ooblastema cells. The rusts may have evolved from the red algae with the loss of the carpogonial branch. In those species where the auxiliary cells are arranged more or less compactly in a palisade, the opportunity for cell fusions between haploid cells would be afforded. The first rusts originating at this time may have completed their life cycles without nuclear fusions, and only later become so adjusted to the new state as to bring to bear the full effect of the auxiliary cell fusion by a nuclear fusion. Cell fusions in the rusts are not sexual fusions, and the cells involved are not sex organs any more than are the anastomoses of hyphae involved when the + and − strains of a heterothallic Coprinus come together. The effects of the cell fusions are just as potent as sexual fusions and perhaps even more so; the substitute process is not necessarily inferior. The Ascomycetes may very well have been evolved from the red algae when the tip of a diploid ooblastema filament bent about and fused with its subterminal cell. With a fusion of two diploid nuclei and a double reduction as proposed by Harper (*8*) the eight

nuclei in the ascus would be accounted for. It is not necessary that a double fertilization including two separate nuclear fusions should occur in every ascomycete to-day. It is conceivable that the inheritance handed down originally from the fusion of diploid nuclei is still manifested in the three divisions in the ascus, even when no nuclear fusions occur. The promycelia of *Caeoma nitens* and *Endophyllum euphorbiae* have four cells in spite of the fact that there have been no nuclear fusions and no reduction divisions.

It is obvious that one can not deny nuclear fusions because he finds in stained preparations two nuclei in every mature spore, unless a large part of the original sowing of the spores is allowed to complete germination and it is found that promycelia and not long germ tubes develop in these controls. On the other hand, one can not maintain that nuclear fusions occur on the basis of finding a large percentage of mature spores with a single nucleus in each spore, unless an equally large percentage of spores in control cultures develop four-celled and not two-celled germ tubes.

The long-cycled and short-cycled Rubus orange rusts grow side by side on blackberries. In certain strains both long-cycled and short-cycled aeciospores are sometimes developed in the same sorus. It is just as essential to provide checks or control cultures in this cytological work as it would be when carrying on infection experiments. This is a relatively easy procedure where spores are dusted on agar in Petri dishes or on cover slips coated with agar. As noted previously, germination studies have been carried on to check all the material used for the cytological studies upon which this paper is based.

In discussing types of promycelia produced by strains of Endophyllum or *Caeoma nitens*, it is assumed that one is referring to germ tubes developed under conditions such that the nuclear processes can go on normally. This means that spores should be germinated as soon as shed naturally. They should be floated on water in moist chambers or dusted on the surface of water agar, and kept in a cool place. Germ tubes that grow under water or down into the agar are not normal, as is generally known.

Sections of telia of the Gymnoconia strain on *Rubus occidentalis* show that nuclear fusions do occur in the teliospore of this form. Any stained preparation of a fairly young sorus shows that the spores are originally binucleate, and that the nuclei fuse very early. An exhaustive search is not necessary to convince one of the facts in this case. The diploid nucleus is only slightly larger than are the haploid nuclei which fuse, enough difference, however, to allow for the fusion, since the volumes would vary as the cubes of their diameters.

It has been pointed out that in *Endophyllum sempervivi* there is a strain sometimes found in France which develops long-cycled true aeciospores. Maire (*14*) has suggested that this strain may belong to a heteroecious long-cycled rust, since he was unable to reinfect the Sempervivum with the spores of the variety *aecidioides*. Disregarding for the moment the obvious fact that the complete story of nuclear behavior in this species has not all been told, and accepting the view that at least in one strain of the rust there are nuclear fusions, it will be asked why there should not be found similar races in *Caeoma nitens* where the nuclei fuse. In addition to the long-cycled form there are certain strains in which the aeciospores are

very regularly binucleate, and their promycelia are very constantly four-celled, so that exceptions to this rule are hard to find. It is in such strains that nuclear fusions should be found if they ever occur. Strains of this type particularly have been studied without the discovery of any evidence of nuclear fusions. Their spores germinate just like they do in *E. euphorbiae-silvaticae*. If the mature spore has two nuclei, the promycelium will be four-celled. The two nuclei entering from the spore divide simultaneously, and it is not until the pairs of sister nuclei have slipped by each other that the pairs become separated by a cross wall. One never finds in this form a two-celled promycelium with one nucleus in each cell. However, uninucleate strains with spores which have arisen without cell fusions produce two-celled promycelia with only one nucleus in each cell. Polynucleate spores give rise to the so-called abnormal germ tubes with variable numbers of cells, depending upon the food supply available and upon the accidents occurring during the distribution of the nuclei.

According to Sappin-Trouffy (*27*) and Dodge (*4*), when the aeciospores of certain species germinate, a septum is often laid down, cutting off that part of the germ tube containing most of the cytoplasm. The empty part of the tube next to the spore may look like a stalk, but it has no such morphological significance. The older and longer the germ tube becomes the more likely will such a septum be found. It is a device for confining the living contents of the cell in a smaller inclosure. Kunkel (*11*) says that even if a promycelium of *Caeoma nitens* has five or more cells (exclusive of what he calls the stalk cell), only four sporidia are developed. This would be natural if a nuclear fusion occurred. The writers have seen any number of cases where more than four sporidia were developed on the same promycelium. The absence of nuclear fusions contributes to the perfect functioning of a spore, no matter how many nuclei it may contain at maturity. The so-called abnormal promycelia with three, five, or more cells are not found in the strains of *Caeoma nitens* whose spores are regularly binucleate when mature.

Moreau's *Endophyllum uninucleatum* is a most unique form of rust. The spores arise without cell fusions and germinate just like a teliospore possessed of a fusion nucleus, producing four-celled promycelia. They go through the form of reduction completely, two divisions and four daughter nuclei. Moreau, who has examined preparations of the uninucleate form of *Caeoma nitens* showing germinating spores and two-celled promycelia, says in a letter to the writers that it is entirely unlike their uninucleate Endophyllum. The Moreaus (*18*), in commenting on Poirault's uninucleate Endophyllum on the red Valerian, say it is exactly like their uninucleate form. Such a conclusion is clearly not warranted, because Poirault (*26*) says distinctly that in his rust the promycelium is regularly two-celled and only two sporidia are developed whereas in *E. uninucleatum* it is claimed the promycelium is four-celled. It should be pointed out, however, that in no place have the Moreaus figured a promycelium of *E. uninucleatum* bearing four sporidia. The binucleate spores of *E. euphorbiae-silvaticae* are even slightly smaller than are those of the *uninucleatum* form. If the latter strain of rust has been derived from the former by simply omitting the cell fusions, it is strange that its spores should be so large. Still more difficult is it to explain why the nucleus should divide twice during germina-

tion, when the nuclei of its parent type divide only once. In *E. centranthi-rubri* the nucleus likewise divides only once during germination. It is certainly interesting to find these cases where, though no nuclear fusions occur in the life cycle, a nucleus goes through the form of reduction so meticulously.

A spore may be uninucleate at its origin, and yet contain several nuclei by the time it is set free. The writers have found that the order of nuclear division and the laying down of cross walls in a germ tube is indicative of the nature of the aeciospore producing it. If the promycelia are slender and have two cells, the spores in the control cultures, if fixed before germination begins, will show only one nucelus when stained. Promycelia with seven or eight cells come from giant polynucleate spores. In *Endophyllum sempervivi* one could tell by the same criterion that the aeciospores had a single nucleus, but he could not tell whether the spore contained a fusion nucleus or had a single nucleus from the time of its origin. The same rule would hold true for *E. euphorbiae-silvaticae*. If a promycelium at maturity has only two cells, and a sporidium is developed from either one or both the cells, the writers hold that the spore from which it developed originally contained a single nucleus. On this basis the two-celled promycelia bearing sporidia figured by the Moreaus (*18, p. 19, fig. 2*) would be interpreted as showing that the spores were originally uninucleate rather than binucleate, as they state. Whether or not the various stages in the development of promycelia in the rusts is correlated throughout the group with nuclear behavior remains to be determined. In cases so far described, when a teliospore containing a fusion nucleus germinates, the median septum is laid down in the promycelium before the second nuclear division is well under way. All such promycelia have a two-celled stage with one nucleus in a cell. The writers believe this to be the best evidence presented by Hoffmann (*9*) and the Moreaus (*18*) for nuclear fusions in *Endophyllum sempervivi*. In cases where a spore which is to form a promycelium has only one nucleus, haploid or diploid, there will then be a two-celled stage of the germ tube with only one nucleus in each cell, so in the early two-celled stage it might be difficult to tell, as noted above, whether the spore had a haploid or a diploid nucleus at the time of germination.

The Moreaus (*18, 19*) seem to have been the first to follow the nuclear behavior in those abnormal aeciospores which contain three or more nuclei. They may be correct in their explanation of their disappearance. Degeneration of supernumerary nuclei is not uncommon. Hoffmann (*9*), however, figures promycelia of the same species with eight sporidia. If no nuclear fusions occurred this could be readily understood. If it is admitted that there can be a fusion of nuclei in pairs in the aeciospore the eight sporidia are also easily accounted for.

Kunkel's Figure 8 (*11*) shows very well the two spindle figures in the promycelium after they have slipped by each other. This could not represent the second divisions of a fusion nucleus as he states, unless it were in a very different type of promycelium development than has been commonly described. After the first division of the fusion nucleus in the germ tube from teliospores and from aeciospores of *Endophyllum sempervivi*, as noted above, a septum is formed between the two daughter nuclei.

In multinucleate cells generally such as oogonia, ascogonia, and asci, and in plasmodia, there is a tendency for all of the nuclei occupying the same cytoplasm to divide at the same time. The crosier or ascus hook, and the clamp connections of the higher basidiomycetes, are admirable devices in the development of which nonsister nuclei are cut off in the new cell after a conjugate nuclear division. The two nuclei in the fusion cell and in the spore initial of the rusts divide conjugately, and the presence of nonsister nuclei in the spore is thus assured. In *Caeoma nitens* and *Endophyllum euphorbiae-silvaticae* the nonsister nuclei that come to lie at the center of the promycelium do not divide conjugately in the full sense of the word as it is now used. Conjugate division now also connotes the cutting off of nonsister nuclei in the new cell, whereas in the two cases just mentioned sister nuclei come to occupy the same cell for a short time or until they are separated by a cross wall. A promycelium from a giant spore will contain several nuclei which may divide simultaneously, but their spindles do not all lie parallel; on the contrary, they may extend in any direction, especially as such promycelia are likely to be rather broad and somewhat branched or irregular in shape.

Since announcement of the discovery that strains of *Caeoma nitens* which have no spermogonia have uninucleate aeciospores (*5*) many further observations have been made, all of which confirm the account given at that time. Beyond the suggestion that the behavior of the rust as it exists in certain plants infected artificially is such as to raise the question of heterothallism, the writers have no further explanation to offer regarding the absence of cell fusions regularly in strains without spermogonia. De Bary (*2*) noted the absence of spermogonia on an isolated plant infected with *Endophyllum sempervivi*, while Maire (*15*) pointed out that the variety *aecidioides* always had a greater abundance of spermogonia than he ever found on the short-cycled type. Maire probably never saw spermogonia of *E. valerianae-tuberosae*. Poirault (*26*) says that it was two years before he was able to find spermogonia on leaves infected with *E. centranthi-rubri*. He thinks that if Maire had made a further search, perhaps he would have found spermogonia in the first species. Both might be right. There very likely are strains which, though possessing uninucleate aeciospores, develop spermogonia regularly, and other strains with uninucleate spores that do not develop spermogonia. Maire says that the single nucleus passes through the pore into the germ tube, but he does not show this. The nucleus divides, and a cross wall is laid down. The lower cell loses its nucleus by karyohexis, or it may divide and then both daughter nuclei degenerate. If the story of what the nuclei do in the aeciospore as it matures and germinates is as clear as the reader may suppose from Maire's description, it would have been more convincing if he had figured some of the real critical stages. It is possible that in some of the material of *E. valerianae-tuberosae* studied by Maire the spores have only a single nucleus from the beginning, just as Poirault claimed for his Endophyllum on a closely related host. If one of the two nuclei in the two spores degenerates, the formation of a two-cell promycelium such as he figured is readily understood when one recalls the promycelium formed by *E. centranthi-rubri* and the uninucleate *Caeoma*

nitens. Maire may be correct in his description of nuclear behavior in *E. valerianae-tuberosae*, but the account certainly needs confirmation.

Olive and Whetzel (*24*) were unable to account for the production of only two spores on the promycelia of *Endophyllum stachytarphetae* and *E. circumscriptum*. A cytological examination of the aecia would no doubt be sufficient to enable one to say whether this was due to the lack of cell fusions as in other uninucleate Endophyllums, or whether, as Lindfors (*13*) claims for *Puccinia arenariae*, the promycelia remain two-celled, even after the second division and two sister nuclei pass up into each sporidium.

In the higher basidiomycetes, such as Coprinus, heterothallism is not a generic peculiarity, although with perhaps rare exceptions it is specific. Absence of spermogonia in *Caeoma nitens* as well as in certain of the Endophyllums may always be correlated with the absence of cell fusions and the production of two-spored promycelia. Other species of Endophyllum may have binucleate aeciospores, as Olive and Whetzel's account also seems to infer, without there being spermogonia; only one of the four species which these investigators studied in Porto Rico had these structures. It also would be interesting to know how the teliospores of *Uromyces rudbeckiana* noted by Olive (*23*) would have germinated. He says this species presented an enigmatical variation, in that all the cells—teliospores as well as the vegetative mycelium—had but one nucleus.

Kursanov (*12*) was unable to infect either Anemone or Sorbus with spores from the strain of *Aecidium leucospermum* having mostly uninucleate aeciospores. He recalls, however, that the possibility of a repeated infection of Anemone by this fungus has been demonstrated by Soppitt (*28*), who obtained the development of aecia a year after inoculation. Kursanov (*12*) suggests that perhaps the frequent existence of uninucleate aeciospores may explain this repeated development of aecia on the first host of a heteroecious rust. While Kursanov admits that it would be interesting from a theoretical standpoint to know the cycle of development of such somatically apogamic rusts as *Endophyllum uninucleatum* (*20*), Olive's (*23*) *Uromyces rudbeckiana*, and the uninucleate forms which he had discovered, yet he thinks such fungi should be regarded as accidental monstrosities without profound significance. Opposed to such a view, it might be said that not only would some of the most puzzling questions from a practical standpoint be answered, but the scheme of rust evolution would be made clearer, if all these "monstrosities" were better understood.

Barclay (*1*) showed that repeating aecia are produced when aeciospores of *Uromyces cunninghamianus* are sowed on the host Jasminum. Barclay's account of this fungus is of special interest. The teliospores are developed in the old aecial cups; spermogonia occur only following infection by sporidia from teliospores. According to Barclay, no such structures are found among the secondary aecia. Just how typical or normal for the species is the mode of aeciospore germination described by Barclay is hard to say. He suggests that his fungus may have certain relationships to the Endophyllums, principally because the aeciospore produces a two-celled promyceliumlike germ tube. Each cell puts out a sterigmatous

branch by means of which, he thinks, the host is infected. No sporidia were ever seen on these long sterigmata. Branched germ tubes from aeciospores and uredospores of several species of rusts have been figured (*27, 4*). The two nuclei in the tube divide precociously, and the cytoplasm is cut off in the outer end by a single septum. In these cases the germ tubes are long and slender. Those of Barclay's Uromyces are short, comparatively thick, and very much like certain two-celled promycelia of an Endophyllum (*20, 2*), or the uninucleate *Caeoma nitens* (*5*)[3] which failed to develop sporidia because of cultural conditions or age of the spores. The failure of the sterigmatous branches to produce sporidia is not proof that the two-celled structures of Barclay's fungus are not real promycelia. The life history of this rust is no doubt more complicated than at first appears.

Presumably Lindfors's (*13, p. 44, fig. 17*) text figure of two-celled mature promycelia of *Puccinia arenariae* was drawn from the same gathering of the rust which he fixed for cytological study. His account of nuclear behavior seems to be conclusive, except at one critical point, viz, he does not show that the two sister nuclei which he finds in each cell actually migrate into the sporidium. Heretofore, whenever a sporidium has been found to contain two nuclei, it has been assumed that the original nucleus of the sporidium had divided. Grove (*7*), as Lindfors pointed out, figured four-celled promycelia for this species. Grove says: "No doubt the future will find this Puccinia divided into several biological races if not into distinct species." Wolf (*29*) has described the development of ascospores of *Podospora anserina*. The ascus has regularly eight nuclei, but in the delimitation of the spores two nuclei are included within each spore membrane. The ascospores of this fungus and the sporidia of *Puccinia arenariae* become binucleate in an analagous manner. The production of only two sporidia on the promycelia of the Endophyllum referred to by Olive and Whetzel may also have been due to a similar process of development.

Endophyllum euphorbiae and *Caeoma nitens* have much in common. Both are systemic, perennial, short-cycled rusts. Their binucleate strains have exactly the same nuclear behavior throughout, and produce the same types of promycelia; both have uninucleate strains. Nevertheless, the two rusts may be widely separated in their phylogeny. It is their general morphological character, rather than their habits of parasitism or nuclear behavior, that points the way of phylogeny.

Two binucleate races of *Caeoma nitens* seem to differ in the size, color, and form of their aecidiospores, and one race seems to have more numerous and more prominent spermogonia. Their sporidia may differ in size, although this feature has not been sufficiently studied to warrant a positive statement on this point.

The two uninucleate races differ at least in one respect. One race develops spermogonia and the other does not, at least not to any great extent.

The outlook for obtaining any striking results in crossing the two binucleate races is not very promising, inasmuch as no nuclear

[3] In this paper as originally published, Plates IV and V were transposed.

fusions occur in the spores of either race. Two sporidia would develop into a rust like one parent, and the other two would give new generations of the other parent. If it should be found, however, that heterothallism enters into the question, the results of infection work may yet be found interesting, especially when one remembers that one of the uninucleate strains lacks the male structures. The production of a binucleate strain through the inauguration of cell fusions again, by introducing two uninucleate strains into the same plant, is not impossible.

When it comes to crossing the long-cycled forms with each of the short-cycled races there are more grounds for expecting real hybridization. Nuclear fusions occur in the teliospore of the long-cycled orange rust, but whether long-cycledness would persist in a hybrid from a cross with a short-cycled form is, of course, still problematical. The differences in spore sizes, color, and form, the relative abundance of spermogonia, the ability to infect the black raspberry (*Rubus occidentalis*), are some of the features or characters which would mark the results of crossing the different races of the Rubus orange rust, *Gymnoconia interstitialis*.

SUMMARY

With respect to the number of nuclei originally contained in the spores as they are cut off, there are three types to be found in different strains of the short-cycle, or *Caeoma nitens*, forms of the Rubus orange rust. Spores of the first type arise after a fusion between two adjacent cells in the sorus primordium; such spores are binucleate from their origin. Spores of the second type arise from uninucleate cells of the primordium, and without fusions. Such spores are thus uninucleate at their origin. The process by which certain spores may become multinucleate and concomitantly giant spores, constituting the third type of spore, the germination of which has been described, may be discussed in a later paper.

It has been shown that in case of spores of the first type—the binucleate spores—there are no nuclear fusions in the spore, which is contrary to what has been claimed heretofore, and, furthermore, there are no nuclear fusions at any time during germination. As a spore begins to form a germ tube, the two nuclei elongate, at first becoming pear-shaped, with the narrow part pointing toward the germ pore. Further elongation and distortion of the nuclei take place as they migrate side by side, or one slightly behind the other, out into the germ tube. Upon shortening and rounding up again, the nuclei come to lie in the middle of the tube, one beyond the other, and not side by side. During the entire process of germination a nucleole is present in each nucleus and is distinctly visible in well-stained preparations. Division of the two nuclei then occurs, usually simultaneously. The spindles may lie more or less parallel, but the division is not in the true sense conjugate. One nucleus may divide somewhat in advance of the other. After the chromatin masses have separated and come to the poles the pairs of reforming sister nuclei move apart or slide by one another and a median septum is laid down. Each cell of the promycelium at this stage contains two sister nuclei. Soon after these nuclei have rounded up, two septa are laid down, so that the mature promycelium consists of four

uninucleate cells. In the teliospore where a nuclear fusion occurs the promycelium has a two-celled stage with only one nucleus in each cell.

The nucleus of the uninucleate type of spores, after having migrated into the germ tube, divides only once, and the mature promycelium consists of two uninucleate cells. Only two sporidia are formed.

In case of the multinucleate spores, there are likewise no nuclear fusions. After the nuclei have passed out into the very large germ tube they divide, ordinarily about the same time. Their spindles lie at various angles in the tube, tending to lie in the general direction of the tube, depending on whether or not the tube is branched. The degeneration of nuclei in such promycelia frequently occurs, and irregular distribution of the daughter nuclei may take place, but, if not, there will be twice as many cells in the mature promycelium as there were nuclei in the mature spore, so that promycelia from trinucleate spores would produce six sporidia.

The formation of a hybrid between a short-cycle strain whose spores are uninucleate and a short-cycle strain with binucleate spores would not be expected to lead to the development of a new form of the rust.

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NUTRITIVE VALUE OF THE PROTEIN IN VOLUNTARY MUSCLE, HEART, LIVER, AND KIDNEY, FROM CATTLE, SHEEP, AND HOGS[1]

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INTRODUCTION

The purpose of this investigation was to determine, as accurately as possible by the method employed, the relative nutritive values of voluntary muscle, liver, kidney, and heart, from cattle, sheep, and hogs, as sources of protein for maintenance and growth in albino rats.

The various edible organs and other by-products of cattle, sheep, and hogs represent a considerable proportion of the food value of each of these classes of animals. Lean meat is of value as a food chiefly as a source of protein, and to a lesser extent as a source of vitamins and mineral matter. The edible organs, also, are of value chiefly on account of their protein, although several of them are rich in vitamins, notably the liver and kidneys. In previous papers (*3*, *4*)[2] the writers have reported the results of investigations concerning the vitamin content of the flesh and organs from cattle, sheep, and hogs, and in this paper they give the results of a study of the nutritive value of the protein from the same sources.

The total crude-protein content of an animal tissue ($N \times 6.25$) can be determined accurately, and by correcting for the presence of certain nonprotein nitrogenous constituents, the percentage of true protein can be estimated fairly well; but even such data do not show the true relative nutritive properties of the protein in different tissues from the same animal or in the same tissue from different animals. The determination of the percentage of each of the more important amino acids throws important light upon the nutritive value of the protein in a tissue, but even such information does not necessarily indicate the true food value of the product analyzed. In order to arrive at a fairly accurate estimate of the relative food values of protein from different sources it is necessary to use the so-called "biological method."

The biological method consists in feeding to man or animals the protein to be tested, or food containing the protein, as the sole source of protein in an otherwise adequate diet. The efficiency with which the different proteins are utilized under certain definite conditions, as measured by intake and outgo of nitrogen, or by maintenance of or by increase in weight, indicates the relative nutritive values of those proteins under those particular conditions. In principle, the biological method is relatively simple, but in practice it is beset with many difficulties, owing to numerous disturbing factors. A critical discussion of this field of research will be found in a recent article by Mitchell (*10*).

[1] Received for publication Sept. 17, 1925; issued June, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 1039.

In attempting to estimate the nutritive values of different proteins, one must consider the several functions which this class of food nutrients performs in the animal body, viz, (1) maintenance or repair of body tissue and as a constituent of internal secretions, (2) growth, (3) reproduction, (4) lactation. It does not necessarily follow that a single protein or the combined proteins in an individual food product will be equally efficient for each of the several purposes named, and for this reason most investigators have determined the biological value of a protein for a particular purpose. A few investigators, however, have attempted to determine the relative values of different proteins for all body functions by a series of feeding experiments with rats extending through several generations. In light of recent developments in this field of research, this method of investigation appears to be open to criticism.

Two general classes of biological methods are employed in studying the nutritive value of proteins—viz, one based on intake and outgo of nitrogen, and the other on nitrogen intake as related to maintenance in weight or to growth. For a discussion of the several methods in each class the reader is referred to the article by Mitchell (*10*). It may be said, however, that both classes of methods are capable of yielding very satisfactory results, although each has very definite limitations. The writers have chosen a method belonging to the second class, and have determined, under certain definite conditions the relation between nitrogen intake and growth in young albino rats. This method is based upon the work of Osborne, Mendel and Ferry (*12*).

PREVIOUS INVESTIGATIONS

This discussion will be confined to those investigations in which one or another of the tissues under examination by the writers constituted the sole source of nitrogen in the diet, since they are not concerned at this time with the supplementary value of the proteins of these tissues for each other or for proteins from other sources.

Thomas (*14*) studied the nutritive value of the nitrogenous compounds in a number of important foodstuffs by a series of nitrogen-balance experiments with himself as the subject. In each experiment the food product under examination constituted the only source of protein in the diet, the energy value being adjusted by means of sugar and fat. He calculated the "biological value" for each product tested, that term indicating the number of parts of body nitrogen replaceable by 100 parts of nitrogen in the food consumed. These data indicate the value of the digestible nitrogen from each source for maintenance purposes.

TABLE I.—*Biological value of nitrogenous compounds in different foods, according to Thomas*

Food product	Biological value	Food product	Biological value
Beef	104.7	Potatoes	78.9
Milk	99.7	Yeast	70.5
Casein	70.1	Spinach	63.8
Fish	94.5	Peas	55.7
Rice	88.3	Wheat flour	39.6
Cauliflower	83.9	Corn meal	29.5
Crabs	79.2		

Osborne and Mendel (*11*) found ox muscle and pig liver to be excellent sources of protein for growth in rats. A ration containing 2.4 per cent nitrogen from dried beef ($2.4 \times 6.25 = 15$ per cent protein), 15 per cent fat, and adequate for growth in other respects, induced normal growth in rats. Similar results were obtained with a ration containing 2.4 per cent nitrogen from pig liver, and 23 per cent fat.

Drummond (*1*) determined the relative nutritive values of the coagulable protein from beef and from several kinds of fish by feeding tests with young rats. The percentage of protein in the rations ranged from 6 to 18, but the food intake of the rats is not reported. It was found that 10 per cent of either beef or fish protein in a ration was not quite sufficient to induce normal growth in rats, but that 15 per cent was ample. It was concluded that the coagulable protein from cod, herring, and salmon possessed nutritive value as high as that derived from beef.

McCollum, Simmonds, and Parsons (*6*) investigated the nutritive value of muscle (lean meat), liver, and kidney from the ox as sources of protein for growth and reproduction in rats. The rations were made up to contain 9 per cent of protein ($N \times 6.25$), adequate quantities of vitamins A and B, and mineral matter. A group of young rats was fed each ration, and in some instances the experiment was carried on with rats from the second and third generations. The food intake of the rats was not reported. The relative nutritive values assigned to muscle, liver, and kidney as sources of protein are based upon the growth, reproduction, and appearance of the rats. In concluding their article, they make the following statement (*6, p. 118*):

> The kidney, liver, and muscle of the ox contain proteins which, when they serve as the sole source of nitrogen, and are fed singly as the sole source of protein, but completely supplemented with respect to all necessary factors other than protein, are shown to possess about the same biological value as those of the wheat kernel.
>
> * * * Kidney proteins appear to have higher biological value than those of the other animal tissues yet studied.

In another publication McCollum (*7, pp. 108, 144*) makes the following statements concerning the nutritive value of certain animal proteins:

> *Proteins of extraordinary value.*—Kidney, liver, and milk proteins stand out as a group of foods containing proteins of unusual value. Among the cereal grains, wheat easily stands first in the quality of its proteins.
>
> As a source of protein for the support of growth the kidney is greatly superior to muscle tissue. Liver appears to fall between these in its value as a source of amino acids.

Mitchell (*8*) reports the results of a series of nitrogen-balance studies with rats to determine the biological value of the protein ($N \times 6.25$) in a number of food products. The term "biological value" is used to indicate the proportion of digestible nitrogen that is assimilated by the body, correction being made for metabolic nitrogen in the feces and for endogenous nitrogen in the urine. The biological values obtained by this method for a number of food products are reported as follows:

TABLE II.—*Biological value of proteins in foods, according to Mitchell*

Food product	Biological value when ration contained—		Food product	Biological value when ration contained—	
	5 per cent protein	8 to 10 per cent protein		5 per cent protein	8 to 10 per cent protein
Veal	97	84	Soybean	73	64
Milk	94	83	Casein	71	
Beef	92	[1] 81	Potato	69	63
Do		[2] 68	Navy beans	29	38
Rice	88		Yeast		67
Oats	82	65	Alfalfa		62
Coconut	77	58	Tankage		33
Corn	73	58			

[1] 8 per cent protein. [2] 10 per cent protein.

In referring to the above data, Mitchell states that too much significance should not be attached to small differences in values, but that differences of 10 or more are probably significant.

In a later paper Mitchell and Carman (*9*) report the biological values obtained for the proteins in several other food products. The proteins in eggs, pork, and wheat were found to have values of 93, 74, and 67, respectively.

PRESENT INVESTIGATION

DESCRIPTION AND PREPARATION OF TISSUES

The beef consisted of round steak from fat steers, the pork of fresh hams from "butcher type" hogs, and the lamb of the fore quarters from heavy fat lambs. The livers, hearts, and kidneys were from the same grades of animals. All the above-named products were purchased in unfrozen condition on the local market. Each product was trimmed as free from fat and connective tissue as practicable, ground fine, mixed with water and toluol, and spread out in a thin layer in shallow pans. These were placed in an oven, where the material was dried in a current of air at a temperature not exceeding 60° C. The tissue became thoroughly air-dry in approximately 24 hours, when it was ground fine, transferred to glass jars, and placed in cold storage until needed. Each lot of dried tissue was analyzed for nitrogen and fat before use in a ration.

COMPOSITION OF RATIONS

With a few exceptions, each tissue was incorporated in each of three rations in such proportions that the rations contained 10, 12.5, and 15 per cent of protein ($N \times 6.25$), respectively, from this source. The only other source of protein in the ration was vitamin B, a commercial product prepared from yeast according to the method of Osborne and Wakeman (*13*). This product, which contained 54.7 per cent protein ($N \times 6.25$), was added to the rations in proportions ranging from 1 to 2 per cent. Vitamin A was supplied in cod-liver oil (1 to 2 per cent), or as an ether extract from egg yolk (5 per cent). The fat content of each ration was adjusted to 10

per cent by the addition of a sufficient quantity of hardened cottonseed oil. Mineral matter was supplied to the extent of 4 per cent as an ash mixture made up according to a formula by Drummond and Watson (*2, p. 237*). The rest of the ration consisted of cassava starch, which was found to be practically free from nitrogen. Each ration was made up in the quantity of 1,000 grams, when needed, and it was stored in covered glass jars.

DESCRIPTION AND CARE OF RATS

The young albino rats used in these experiments were raised in the animal laboratory of the Bureau of Animal Industry, from healthy vigorous stock. Litters of more than 8 rats were reduced to that

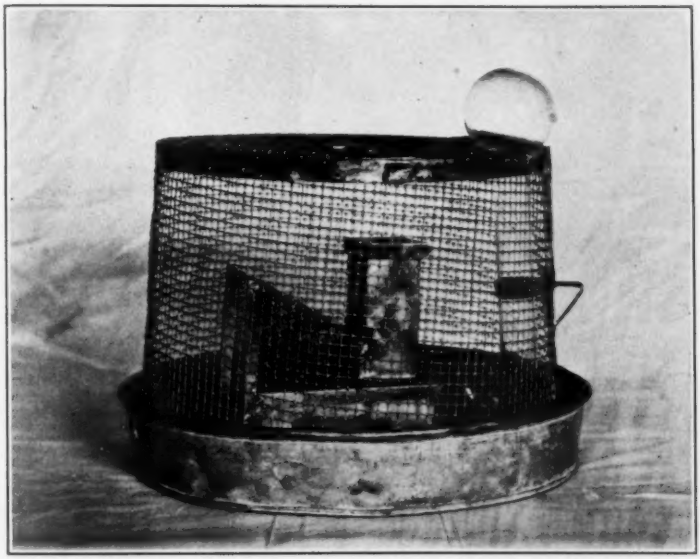

FIG. 1.—Rat cage, equipped with self-feeder and drinking fountain

number on the day of birth, and litters comprising fewer than 6 rats were discarded. Only young rats that weighed at least 40 grams each within 30 days from birth were used for experimental purposes. The breeding rats were fed a ration of uniform composition throughout these experiments.

Each experimental ration was fed to a group of 4 to 8 rats during a total period of 60 days, but the experimental data are tabulated for two periods, viz, (1) the first 30 days, and (2) for the entire 60 days. With few exceptions each ration was fed to a group composed of an equal number of male and female rats. Each rat was kept in an individual cage (fig. 1) and was supplied with water in a glass fountain and with its ration in a self-feeder (fig. 2). In the earlier experiments the rectangular type of feeder was used, but in the later tests an improved feeder of cylindrical shape was sub-

stituted (*5*). The rats were cared for daily, except Sunday, the blotting paper in the bottom of the cage being changed and any feed that had been pulled out of the feeders was replaced. The rats were weighed at the start of the test and regularly thereafter on Tuesday and Friday mornings. The self-feeder containing the ration was weighed when placed in the cage and again when nearly empty, the loss in weight indicating feed consumed. Cages, feeders, and drinking vessels were cleaned and sterilized at regular weekly intervals.

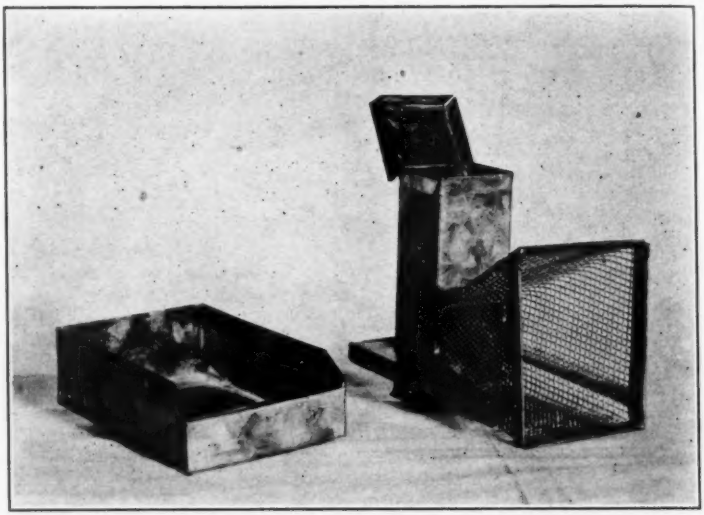

FIG. 2.—Self-feeder for rats

RELATIVE NUTRITIVE VALUE OF PROTEIN IN SEVERAL ANIMAL TISSUES

The results of the feeding experiments with the various animal tissues have been calculated and tabulated to show the result obtained with each rat, as well as the average result for the group of rats fed each ration, but it was found that the detailed results were so extensive as to make their publication undesirable. For this reason, only the average results will be presented.

THIRTY-DAY EXPERIMENTS

As regards the data presented in Table III, judgment of the relative nutritive values of the protein from the several tissues should be based chiefly upon the relation between feed and protein consumed, and the gain in weight of the rats, although the total gain in weight is of some importance also. The average data are given for each sex, but for the sake of brevity the discussion will be limited, for the most part, to the average data for both sexes. Although the results for the two sexes do not always run parallel, it is believed that the

average data for the male and female rats furnish the most satisfactory basis for comparison, on account of the larger number of rats involved.

Referring to the average total gain in weight, it will be noted that the largest gain (86 grams) was made by the rats fed ox muscle and hog liver, respectively, and the smallest gain (61 grams) by those fed ox liver. The gain made by the rats fed ox muscle and hog liver, respectively, was 41 per cent greater than that made by those fed ox liver. The gains made by the other groups of rats ranged from 85 grams for those fed ox hearts to 73 grams for those fed hog hearts, or a difference of 16 per cent in favor of the former.

TABLE III.—*Average* [1] *growth values obtained with rats for muscle, heart, liver, and kidney from the ox, sheep, and hog, when the rations contained 10 per cent protein, and the duration of the test was 30 days*

Kind of tissue	Sex of rats	Number of rats	Gain in weight in 30 days	Total intake		Intake per gram gain in weight		Gain in weight		Average daily consumption per gram body weight	
				Feed	Protein	Feed	Protein	Per gram of feed	Per gram of protein	Feed	Protein
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Ox muscle	Male	4	95	302	30.2	3.2	0.32	0.31	3.15	0.1127	0.0113
Do	Female	4	76	252	25.2	3.4	.34	.30	3.00	.1059	.0106
Average			86	277	27.7	3.3	.33	.31	3.08	.1093	.0109
Hog muscle	Male	4	84	281	28.1	3.4	.34	.30	2.96	.1070	.0107
Do	Female	4	73	279	27.9	3.8	.38	.26	2.62	.1115	.0112
Average			79	280	28.0	3.6	.36	.28	2.79	.1093	.0109
Sheep muscle	Male	4	85	274	27.4	3.2	.32	.31	3.12	.1095	.0110
Do	Female	4	71	257	25.7	3.7	.37	.28	2.76	.1082	.0108
Average			78	266	26.6	3.5	.35	.30	2.94	.1089	.0109
Ox heart	Male	5	86	275	27.5	3.2	.32	.31	3.11	.1119	.0112
Do	Female	3	83	270	27.0	3.2	.32	.31	3.09	.1072	.0107
Average			85	273	27.3	3.2	.32	.31	3.10	.1096	.0110
Hog heart	Male	4	81	265	66.5	3.3	.33	.31	3.04	.1103	.0110
Do	Female	4	65	234	23.4	3.6	.36	.28	2.77	.1073	.0107
Average			73	250	25.0	3.5	.35	.30	2.91	.1088	.0109
Ox liver	Male	7	70	243	24.3	3.6	.36	.28	2.82	.1040	.0104
Do	Female	1	52	207	20.7	4.0	.40	.25	2.51	.1062	.0106
Average			61	225	22.5	3.8	.38	.27	2.67	.1050	.0105
Hog liver	Male	4	103	292	29.2	2.9	.29	.36	3.54	.1073	.0107
Do	Female	4	69	241	24.1	3.5	.35	.29	2.88	.1102	.0110
Average			86	267	26.7	3.2	.32	.33	3.21	.1088	.0109
Sheep liver	Male	4	82	257	25.7	3.2	.32	.32	3.18	.1016	.0102
Do	Female	4	78	277	27.7	3.6	.36	.29	2.82	.1108	.0111
Average			80	267	26.7	3.4	.34	.31	3.00	.1062	.0106
Ox kidney	Male	4	78	260	26.0	3.4	.34	.30	3.00	.1055	.0106
Do	Female	4	78	288	28.8	3.8	.38	.28	2.71	.1151	.0115
Average			78	274	27.4	3.6	.36	.29	2.86	.1103	.0110
Hog kidney	Male	5	88	281	28.1	3.2	.32	.32	3.18	.1089	.0109
Do	Female	3	64	220	22.0	3.5	.35	.29	2.90	.1020	.0102
Average			76	251	25.1	3.4	.34	.31	3.04	.1055	.0106

[1] In this and succeeding tables the average for the two sexes is the average of the averages for the males and females, respectively.

The feed intake per gram of gain in weight was largest for the rats fed ox liver (3.8 grams), and smallest for those fed ox heart and hog liver, respectively (3.2 grams).

The greatest gain in weight per gram of protein consumed was made by the rats fed hog liver (3.21 grams), and the smallest gain by those fed ox liver (2.67 grams), a difference of 20 per cent in favor of hog liver. If these two products are excepted, the values for the others range from 3.10 grams for ox hearts to 2.79 grams for hog muscle, or a difference of only 11 per cent in favor of ox hearts.

TABLE IV.—*Average growth values obtained with rats for muscle, heart, liver, and kidney from the ox, sheep, and hog, when the rations contained 10 per cent protein, and the duration of the test was 60 days*

Kind of tissue	Sex of rats	Number of rats	Gain in weight in 60 days	Total intake		Intake per gram gain in weight		Gain in weight	
				Feed	Protein	Feed	Protein	Per gram of feed	Per gram of protein
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Ox muscle	Male	4	170	667	66.7	3.9	0.39	0.25	2.55
Do	Female	4	114	523	52.3	4.6	.46	.22	2.18
Average			142	595	59.5	4.3	.43	.24	2.37
Hog muscle	Male	4	137	554	55.4	4.1	.41	.25	2.46
Do	Female	4	106	531	53.1	5.0	.50	.20	2.02
Average			122	543	54.3	4.6	.46	.23	2.24
Sheep muscle	Male	4	152	611	61.1	4.1	.41	.25	2.48
Do	Female	4	103	534	53.4	5.2	.52	.19	1.94
Average			128	573	57.3	4.7	.47	.22	2.21
Ox heart	Male	5	168	639	63.9	3.8	.38	.26	2.64
Do	Female	3	117	520	52.0	4.5	.45	.22	2.24
Average			143	580	58.0	4.2	.42	.24	2.44
Hog heart	Male	4	149	596	59.6	4.0	.40	.25	2.49
Do	Female	4	98	481	48.1	4.9	.49	.21	2.06
Average			124	539	53.9	4.5	.45	.23	2.28
Ox liver	Male	7	126	543	54.3	4.3	.43	.23	2.31
Do	Female	1	91	488	48.8	5.4	.54	.19	1.86
Average			109	516	51.6	4.9	.49	.21	2.09
Hog liver	Male	4	171	673	67.3	4.0	.40	.25	2.53
Do	Female	4	102	497	49.7	5.0	.50	.20	2.02
Average			137	585	58.5	4.5	.45	.23	2.28
Sheep liver	Male	4	156	598	59.8	3.8	.38	.26	2.61
Do	Female	4	119	564	56.4	4.8	.48	.21	2.10
Average			138	581	58.1	4.3	.43	.24	2.36
Ox kidney	Male	4	135	557	55.7	4.2	.42	.24	2.42
Do	Female	4	110	539	53.9	5.0	.50	.20	2.02
Average			123	548	54.8	4.6	.46	.22	2.22
Hog kidney	Male	5	159	622	62.2	3.9	.39	.26	2.56
Do	Female	3	86	471	47.1	5.6	.56	.18	1.18
Average			123	547	54.7	4.8	.48	.22	2.19

Sixty-Day Experiments

From Table IV it will be noted that the greatest average gain in weight was made by the rats fed ox hearts (143 grams), and the smallest gain by those fed ox liver (109 grams), a difference of 31 per cent in favor of the former. The gains made by the other groups of rats ranged from 142 grams for those fed ox muscle to 122 grams for those fed hog muscle, or a difference of 16 per cent in favor of ox muscle.

The feed intake for each gram of gain in weight made by the rats ranged from 4.2 grams for ox hearts, to 4.9 grams for ox liver.

The gain in weight per gram of protein consumed was greatest for the rats fed ox heart (2.44 grams), and smallest for those fed ox liver (2.09 grams), a difference of 17 per cent in favor of the ox heart. The values for the other tissues range from 2.19 grams for hog kidneys, to 2.37 for ox muscle, or a difference of only 8 per cent.

The variations in the results obtained during the 60-day test are smaller than those in the results obtained during the 30-day test. This was to have been expected, of course, on account of the decreasing requirement of the rat for protein as it increases in weight.

RELATIVE NUTRITIVE VALUES OF ANIMAL TISSUES WHEN RATIONS CONTAINED 12.5 PER CENT PROTEIN

Thirty-Day Experiments

From Table V it will be noted that the average gain in weight made by each of the several groups of rats ranged from 87 grams for those fed ox kidney to 101 grams for those fed sheep liver, or a difference of 16 per cent in favor of the sheep liver. This is to be contrasted with a difference of 41 per cent in the gains made by the rats fed the rations containing 10 per cent protein for a period of 30 days.

The feed intake for each gram of gain in weight ranged from 2.7 grams for the rats fed hog kidney to 3.4 grams for those fed hog liver.

The greatest gain in weight per gram of protein consumed was made by the rats fed hog kidney (3.01 grams), and the smallest gain by those fed hog liver (2.46 grams), a difference of 22 per cent in favor of hog kidney.

Taking into consideration the several factors involved, it appears that hog kidney and hog heart had somewhat higher values as sources of protein for growth than did the other tissues, when the rations contained 12.5 per cent protein and the duration of the test was 30 days. The differences in the values obtained for the products other than hog heart and hog kidney are so small as to have no significance.

TABLE V.—*Average growth values obtained with rats for muscle, heart, liver, and kidney from the ox, sheep, and hog, when the rations contained 12.5 per cent protein, and the duration of the test was 30 days*

Kind of tissue	Sex of rats	Number of rats	Gain in weight in 30 days	Total intake		Intake per gram gain in weight		Gain in weight		Average daily consumption per gram body weight	
				Feed	Protein	Feed	Protein	Per gram of feed	Per gram of protein	Feed	Protein
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Ox muscle	Male	3	107	313	39.1	2.9	0.37	0.34	2.73	0.1035	0.0129
Do	Female	5	69	242	30.5	3.6	.45	.28	2.27	.0985	.0123
Average			88	278	34.8	3.3	.41	.31	2.50	.1010	.0126
Hog muscle	Male	2	108	308	38.5	2.9	.36	.35	2.82	.1072	.0134
Do	Female	2	80	274	34.3	3.5	.43	.29	2.32	.0990	.0124
Average			94	291	36.4	3.2	.40	.32	2.57	.1031	.0129
Sheep muscle	Female	4	78	274	34.3	3.5	.44	.29	2.27	.1113	.0139
Ox heart	Male	1	114	307	38.4	2.7	.34	.37	2.97	.1089	.0136
Do	Female	3	77	267	33.3	3.5	.44	.29	2.31	.1090	.0136
Average			96	287	35.9	3.1	.39	.33	2.64	.1080	.0136
Hog heart	Male	3	114	286	35.8	2.5	.32	.40	3.21	.1023	.0128
Do	Female	1	73	217	27.1	3.0	.37	.34	2.69	.0924	.0116
Average			94	252	31.5	2.8	.35	.37	2.95	.0974	.0122
Ox liver	Male	2	121	325	40.7	2.7	.34	.37	2.97	.0972	.0122
Do	Female	2	65	253	31.7	3.9	.49	.26	2.05	.0985	.0123
Average			93	289	36.2	3.3	.42	.32	2.51	.0976	.0123
Hog liver	Male	3	111	304	38.0	2.8	.35	.36	2.91	.0983	.0123
Do	Female	1	64	254	31.8	4.0	.50	.25	2.01	.1052	.0132
Average			88	279	34.9	3.4	.43	.31	2.46	.1018	.0128
Sheep liver	Male	2	117	335	41.9	2.9	.36	.35	2.78	.1155	.0144
Do	Female	2	85	275	34.4	3.3	.41	.31	2.46	.1086	.0136
Average			101	305	38.2	3.1	.39	.33	2.62	.1121	.0140
Ox kidney	Male	2	84	256	32.1	3.2	.39	.33	2.58	.0977	.0122
Do	Female	2	89	274	34.2	3.1	.39	.33	2.59	.1081	.0135
Average			87	265	33.2	3.2	.39	.33	2.59	.1029	.0129
Hog kidney	Male	2	109	270	33.7	2.5	.31	.40	3.22	.0968	.0121
Do	Female	2	86	244	30.6	2.9	.36	.35	2.79	.0988	.0124
Average			98	257	32.2	2.7	.34	.38	3.01	.0978	.0123

SIXTY-DAY TESTS

In Table VI are summarized the results of the 60-day feeding experiments with rations containing 12.5 per cent protein. The average total gain in weight made by each of the groups of rats varied within comparatively narrow limits, ranging from 158 grams for those fed ox heart to 139 grams for those fed ox liver, or a difference of only 13.7 per cent in favor of the ox heart.

The feed intake per gram of gain in weight ranged from 3.8 grams for the rats fed hog kidneys to 4.5 grams for those fed ox muscle and hog liver, respectively.

The gain in weight per gram of protein consumed ranged from 2.18 grams for the rats fed hog heart to 1.84 grams for those fed hog liver, or a difference of 18 per cent in favor of the hog heart.

The results of this series of experiments are very similar to those obtained with the same rations during the 30-day test, as regards relative nutritive values of the proteins from the several tissues. Hog heart and hog kidney seemed to have somewhat higher values than the other tissues.

TABLE VI.—*Average growth values obtained with rats for muscle, heart, liver, and kidney from the ox, sheep, and hog, when the rations contained 12.5 per cent protein, and the duration of the test was 60 days*

Kind of tissue	Sex of rats	Number of rats	Gain in weight in 60 days	Total intake		Intake per gram gain in weight		Gain in weight	
				Feed	Protein	Feed	Protein	Per gram of feed	Per gram of protein
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Ox muscle	Male	3	194	718	89.8	3.8	0.47	0.26	2.14
Do	Female	5	101	509	63.6	5.1	.64	.20	1.58
Average			148	614	76.7	4.5	.56	.23	1.86
Hog muscle	Male	2	174	668	83.5	3.9	.49	.26	2.08
Do	Female	2	117	555	69.4	4.8	.60	.21	1.69
Average			146	612	76.5	4.4	.55	.24	1.89
Sheep muscle	Female	4	113	556	69.4	4.9	.62	.20	1.63
Ox heart	Male	1	201	681	85.1	3.4	.42	.30	2.36
Do	Female	3	114	519	64.9	4.5	.57	.22	1.76
Average			158	600	75.0	4.0	.50	.26	2.06
Hog heart	Male	3	190	651	81.4	3.8	.43	.29	2.33
Do	Female	1	121	478	59.8	4.0	.49	.25	2.02
Average			156	565	70.6	3.9	.46	.27	2.18
Ox liver	Male	2	179	656	82.0	3.7	.46	.27	2.18
Do	Female	2	98	499	62.4	5.1	.64	.20	1.57
Average			139	578	72.2	4.4	.55	.24	1.88
Hog liver	Male	3	186	679	84.9	3.7	.46	.27	2.18
Do	Female	1	95	508	63.5	5.3	.67	.19	1.50
Average			141	594	74.2	4.5	.57	.23	1.84
Sheep liver	Male	2	185	735	91.9	3.8	.50	.26	2.01
Do	Female	2	120	553	69.2	4.7	.58	.22	1.73
Average			153	644	80.6	4.3	.54	.24	1.87
Ox kidney	Male	2	161	590	73.8	3.8	.47	.27	2.18
Do	Female	2	126	596	70.3	4.5	.56	.22	1.79
Average			144	576	72.1	4.2	.52	.25	1.98
Hog kidney	Male	2	183	607	75.9	3.4	.42	.31	2.41
Do	Female	2	120	502	62.7	4.2	.53	.24	1.90
Average			152	555	69.3	3.8	.48	.28	2.16

RELATIVE NUTRITIVE VALUES OF ANIMAL PROTEINS WHEN RATIONS CONTAINED 15 PER CENT PROTEIN

THIRTY-DAY EXPERIMENTS

Only 7 of the 10 animal tissues previously examined were tested in this experiment, and one was fed to female rats only. In Table VII it will be noted that the average total gain in weight for the

rats of both sexes ranged from 104 grams for the rats fed ox liver to 82 grams for those fed sheep muscle, or a difference of 27 per cent in favor of the ox liver. The gains made by the other groups of rats ranged from 101 grams for those fed ox heart to 89 grams for those fed hog muscle, or a difference of 13 per cent in favor of the former.

The feed intake per gram gain in weight ranged from 2.8 grams for the rats fed ox liver to 3.3 grams for those fed sheep muscle.

The gain in weight per gram of protein consumed varied from 2.40 grams for the rats fed ox liver to 2.06 grams for those fed sheep muscle, or a difference of 16.5 per cent in favor of the ox liver. If sheep muscle is excepted, the variation in the values of the other tissues amounts to only 9 per cent.

The results of this series of tests appear to indicate that sheep muscle had a somewhat lower nutritive value than the other tissues in this group, but, considering the limited number of rats used to test each tissue, it is doubtful whether the difference is great enough to be significant.

Sixty-Day Experiments

In Table VIII it will be noted that the greatest average gain in weight was made by the rats fed the ration containing ox heart (165 grams), and the smallest gain by those fed sheep muscle (142 grams), or a difference of 16 per cent in favor of the ox heart.

Table VII.—*Average growth values obtained with rats for muscle, heart, and liver from the ox, sheep, and hog, when the rations contained 15 per cent protein, and the duration of the test was 30 days*

Kind of tissue	Sex of rats	Number of rats	Gain in weight in 30 days	Total intake		Intake per gram gain in weight		Gain in weight		Average daily consumption per gram body weight	
				Feed	Protein	Feed	Protein	Per gram of feed	Per gram of protein	Feed	Protein
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Ox muscle	Male	2	107	300	45.0	2.8	0.42	0.36	2.38	0.0977	0.0147
Do	Female	2	84	254	38.1	3.1	.46	.33	2.20	.0981	.0147
Average			96	277	41.6	3.0	.44	.35	2.29	.0979	.0147
Hog muscle	Male	2	99	277	41.5	2.8	.42	.36	2.39	.0954	.0143
Do	Female	2	78	242	36.3	3.1	.47	.32	2.14	.0959	.0144
Average			89	260	38.9	3.0	.45	.34	2.27	.0957	.0144
Sheep muscle	Male	1	85	271	40.7	3.2	.48	.31	2.09	.1013	.0152
Do	Female	3	79	259	38.9	3.3	.49	.30	2.02	.1009	.0151
Average			82	265	39.8	3.3	.49	.31	2.06	.1011	.0152
Ox heart	Male	2	125	326	48.9	2.6	.39	.38	2.56	.1047	.0157
Do	Female	2	77	245	37.7	3.2	.49	.32	2.04	.1017	.0153
Average			101	286	43.3	2.9	.44	.35	2.30	.1032	.0155
Ox liver	Male	3	120	310	46.6	2.6	.39	.38	2.57	.0940	.0141
Do	Female	1	88	264	39.6	3.0	.45	.33	2.22	.0943	.0141
Average			104	287	43.1	2.8	.42	.36	2.40	.0942	.0141
Hog liver	Male	2	114	305	45.7	2.7	.41	.37	2.48	.0950	.0143
Do	Female	2	66	227	34.1	3.4	.52	.29	1.94	.0933	.0140
Average			90	266	39.9	3.1	.47	.33	2.21	.0942	.0142
Sheep liver	Female	4	90	289	43.4	3.3	.49	.31	2.07	.1074	.0161

TABLE VIII.—*Average growth values obtained with rats for muscle, heart, and liver from the ox, sheep, and hog, when the rations contained 15 per cent protein, and the duration of the test was 60 days*

Kind of tissue	Sex of rats	Number of rats	Gain in weight in 60 days	Total intake		Intake per gram gain in weight		Gain in weight	
				Feed	Protein	Feed	Protein	Per gram of feed	Per gram of protein
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Ox muscle	Male	2	170	669	100.2	3.9	0.60	0.26	1.7
Do	Female	2	125	545	81.8	4.4	.66	.23	1.53
Average			148	607	91.0	4.2	.63	.25	1.62
Hog muscle	Male	2	175	624	93.6	3.6	.54	.28	1.87
Do	Female	2	118	577	86.6	4.9	.74	.21	1.37
Average			147	601	90.1	4.3	.64	.25	1.62
Sheep muscle	Male	1	164	590	88.5	3.6	.54	.28	1.85
Do	Female	3	120	534	80.1	4.5	.68	.22	1.47
Average			142	562	84.3	4.1	.61	.25	1.66
Ox heart	Male	2	205	719	107.9	3.6	.53	.29	1.90
Do	Female	2	124	518	77.7	4.2	.63	.24	1.59
Average			165	619	92.8	3.9	.58	.27	1.75
Ox liver	Male	3	195	638	95.7	3.3	.49	.31	2.04
Do	Female	1	115	455	68.3	4.0	.59	.25	1.68
Average			155	547	82.0	3.7	.54	.28	1.86
Hog liver	Male	2	187	683	102.4	3.7	.55	.28	1.83
Do	Female	2	98	481	72.1	5.0	.76	.21	1.35
Average			143	582	87.3	4.4	.66	.25	1.59
Sheep liver	Female	4	122	582	87.3	4.8	.72	.21	1.40

The feed consumption per gram of gain in weight ranges from 3.7 grams for the rats fed ox liver to 4.4 for those fed hog liver.

The greatest gain in weight per gram of protein consumed was made by the rats fed ox liver (1.86 grams), and the smallest gain by those fed hog liver (1.59 grams), a difference of 17 per cent in favor of the ox liver.

The results of the 60-day feeding test with rations containing 15 per cent of protein from each of 6 animal tissues, respectively, seem to indicate that ox liver had a somewhat higher value as a source of protein for maintenance and growth than the other tissues, but, considering the fact that each ration was tested with only 4 rats, it is possible that this difference is within the limit of experimental error.

RELATIVE NUTRITIVE REQUIREMENTS OF MALE AND FEMALE RATS

In Tables III to VIII, inclusive, it will be noted that, with relatively few exceptions, very different results were obtained from the male rats than from the female. With two exceptions, the male rats made a greater gain in weight per gram of protein consumed, and required less feed for each gram of gain in weight, than did the female rats getting the same ration. The two exceptions are: (1) The

rats fed the ration containing 10 per cent of ox heart protein for a period of 30 days (Table III), and (2) those fed the ration containing 12.5 per cent of ox kidney protein for a like period (Table V). In both of these instances, practically the same results were obtained from the male rats as from the female.

An explanation for the difference in the results obtained from the two sexes is not hard to find. In Tables III to VIII, inclusive, it will be observed that, with only a few exceptions, the male rats made considerably greater gains in weight than did the females getting the same ration, the differences being greater for the 60-day than for the 30-day tests. This indicates that the feed was utilized more efficiently for growth by the faster-growing rats, the males, than by the slower-growing rats, the females. This is in accordance with the well-known fact that the faster-growing animal utilizes its feed more efficiently for growth than does the slower-growing one, chiefly for the reason that the latter requires a larger proportion of its feed for maintenance than does the former.

This explanation is supported by the fact that in two instances where the male rats made approximately the same gain in weight as did the female, both sexes utilized the ration with practically equal efficiency for growth (rats fed ox heart, Table III, and those fed ox kidney, Table V). Thus, rate of growth, rather than sex, seems to determine the efficiency with which the rat utilizes its feed for growth.

In another instance, however (ox kidney ration, Table III), the male and female rats made the same gain in weight, but the latter consumed appreciably more feed and consequently utilized their ration less efficiently for growth.

However, the preponderance of evidence supports the view that the female rats utilized their feed less efficiently for growth than did the male rats, because of the difference in the rate of growth of the two sexes.

The practical significance of these observations, as related to the use of male and female rats for the determination of the relative nutritive values of proteins, is apparent.

The fact that in every instance the rats of both sexes utilized their feed much more efficiently for growth during the 30-day test than during the entire period of 60 days is in harmony with the experience of practical feeders and students of nutrition, that the larger animals require less feed per pound of gain early in the growing period than later.

SUMMARY OF RESULTS

In this paper are reported the results of a series of feeding experiments with young albino rats to determine the relative values for maintenance and growth of the protein in voluntary muscle, heart, liver, and kidney from cattle, sheep, and hogs.

Protein was fed at three levels of intake, viz, 10, 12.5, and 15 per cent, and the rations were prepared so as to have practically the same energy value.

When protein was fed at the 10 per cent level for a period of 30 days, hog liver had a somewhat higher value and ox liver a slightly lower value than any of the other tissues; but the differences in the

values obtained for the several tissues were less marked when the duration of the experiment was 60 days.

When protein was fed at the 12.5 per cent level, hog heart and hog kidney seemed to have somewhat higher values than the other tissues, for both the 30 and the 60 day experiments.

Only ox, hog, and sheep muscle, ox heart, and ox, hog, and sheep liver were fed in rations containing 15 per cent protein, and the differences in the values obtained do not seem large enough to be significant.

The male rats were found to utilize protein much more efficiently for growth than the female rats. This is explained by the more rapid growth of the male rats, resulting in a smaller proportionate requirement of protein for maintenance as compared with that needed for growth than was the case with the slower-growing female rats.

Each ration was utilized much more efficiently for growth during the 30-day than during the 60-day experiment. This is due to the increasing requirement of the rat for feed for maintenance, as compared with that required for growth, as it increases in weight.

As regards the probable relative nutritive values of voluntary muscle, heart, liver, and kidney from the ox, sheep, and hog, as sources of protein in the human dietary, it seems likely that when the diet contains an adequate amount of protein these tissues will have approximately the same value for maintenance and growth.

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DETERMINATION OF THE PERCENTAGE OF INFESTATION BY THE HESSIAN FLY, PHYTOPHAGA DESTRUCTOR SAY [1]

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INTRODUCTION

At the time of a symposium on methods of estimating insect abundance and damage [2] held at the regular meeting of the American Association of Economic Entomologists, at Cincinnati, a series of studies was under way by which it was intended to supplement in more detail the contribution on the Hessian fly. The present paper is the first of a series in which it is hoped to present a partial solution of some of the questions which have arisen in a statistical study of this problem.

Percentage of infestation is one of the determinations most often made in connection with control and other studies of the Hessian fly. Since many conclusions are drawn from these determinations, it seems appropriate and desirable that the method of obtaining such information should receive careful attention.

THE PROBLEM

In the determination of percentage of infestation of wheat by the Hessian fly, two points have been a continuous source of difficulty: (1) What are the limitations of determinations made from samples selected in various ways; (2) what are the limitations of samples containing various numbers of culms?

DISCUSSION

At Centralia, Ill., in the fall of 1923, samples of wheat culms were taken and grouped in four series, each of which contained 100 samples, each sample being made up of 100 culms taken as shown below:

Series 1, 100 samples, each of 100 culms; 1 culm from each of 100 random places.

Series 2, 100 samples, each of 100 culms; 20 culms from each of 5 random places.

Series 3, 100 samples, each of 100 culms; 50 culms from each of 2 random places.

Series 4, 100 samples, each of 100 culms; 100 culms, all from 1 random place.

[1] Received for publication Nov. 3, 1925; issued June, 1926. This is the first of a series of papers in which is discussed the application of statistical methods to the study of the Hessian fly.

[2] MARLATT, C. L., GOSSARD, H. A., HYSLOP, J. A., FELT, E. P., WEBSTER, R. L., FLINT, W. P., HINDS, W. E., and HOWARD, L. O. [REMARKS ON A SYMPOSIUM ON "METHODS OF ESTIMATING INSECT ABUNDANCE AND DAMAGE."] Jour. Econ. Ent. 17 : 207-214. 1924.

From the results obtained by the examination of these samples, the standard deviation of each method was determined. Since the method of taking 20 culms from each of five places is the one in use at the United States Entomological Field Laboratory, West Lafayette, Ind., the determination of its standard deviation will be given as an example. Standard deviation in this case may be defined as the square root of the mean of the squares of the deviations from the arithmetical mean.

The standard deviation (σ) of the method in which 20 culms are taken from each of five places (see Table I) is determined according to the formula

$$\sigma = \sqrt{\frac{\Sigma f x^2}{n}},$$

in which σ designates the standard deviation, f the frequency, x the deviation from the mean, n the number of samples examined, and Σ indicates summation.

TABLE I.—*Frequency table showing the percentage determinations of infestation by the Hessian fly of 100 wheat samples, each of 100 culms, and the determination of the standard deviation of the method "20 culms from each of 5 random places."*

Infestation (*V*)	Frequency (*f*)	*fV*	Deviation from mean (57) *x*	x^2	fx^2
Per ct.					
44	1	44	13	169	169
45	1	45	12	144	144
46	1	46	11	121	121
48	3	144	9	81	243
49	2	98	8	64	128
50	8	400	7	49	392
51	8	408	6	36	288
52	2	104	5	25	50
53	5	265	4	16	80
54	2	108	3	9	18
55	7	385	2	4	28
56	12	672	1	1	12
57	4	228	0	0	0
58	7	406	1	1	7
59	9	531	2	4	36
60	6	360	3	9	54
61	8	488	4	16	128
63	4	252	6	36	144
64	3	192	7	49	147
65	1	65	8	64	64
66	1	66	9	81	81
68	2	136	11	121	242
69	1	69	12	144	144
70	1	70	13	169	169
77	1	77	20	400	400
----------	100	5,659	----------	----------	3,289

$$\sigma = \sqrt{\frac{3,289}{100}} = 5.73$$

The infestation of the samples varied from 44 per cent to 77 per cent; the several percentages (V) are shown in the first column of Table I. In the second column is listed the number (f) of times a

sample was found with the corresponding percentage of infestation; in other words, the frequency of occurrence. For instance, one was found with a 44 per cent infestation, one with 45 per cent, twelve with 56 per cent, and so on. By multiplying the corresponding figures in the first and second columns, the third column (fV) is obtained, and adding these products, the result is the sum of the percentages of infestation of the 100 samples. Dividing by 100, the number of samples, the arithmetical mean of approximately 57 per cent is obtained. In the fourth column is given the difference (x) between 57 per cent and the various percentages in the first column, or, in other words, their deviation from the arithmetical mean. Since the definition calls for the mean of the squares of these deviations, the fifth column contains the squares (x^2) of the numbers in the fourth column, and in the sixth is given the products (fx^2) of the several deviations squared, each multiplied by the number of times it occurs, as indicated in the second column. The sum of these products divided by 100 (the number of samples) gives the mean square deviation 32.89, the square root of which is 5.73, the standard deviation. The formula for this determination therefore can be written

$$\text{Standard deviation, } \sigma, = \sqrt{\frac{\Sigma fx^2}{n}} \text{ (Formula 1),}$$

where Σ indicates summation, and n is the number of samples in this case, or, to use the more general term, the number of variates.

In a similar manner, using the same formula, the standard deviations of the other series were determined. So far no theory is involved, it being simply a case of straightforward arithmetic. It has been found, however, that in cases of this kind the standard deviation, σ, of a normal distribution can be determined by the formula[1]

$$\text{Standard deviation, } \sigma, = \sqrt{\frac{pq}{n}} \text{ (Formula 2),}$$

where p designates the percentage of infested culms, q the percentage of uninfested culms, and n the number of culms examined. Substituting in this generally accepted formula the standard deviation (σ) of a theoretical representative sample of 100 culms of a normal distribution in a field of 57 per cent infestation,

$$\sigma = \sqrt{\frac{57 \times 43}{100}} = 4.95.$$

Since the dispersion of a set of observations around their arithmetical mean is measured by the standard deviation, it seems that a relative comparison of the methods might be made by comparing their respective standard deviations. Before making this comparison, however, it would be well to compute the probable error of the standard deviations. This can be done by the formula

$$PE_\sigma = \frac{0.6745\sigma}{\sqrt{2n}} \text{ (Formula 3).}$$

[1] For more detailed information regarding the formulas used in this discussion, reference is made to: KELLEY, T. L., STATISTICAL METHOD. 390 p., illus. New York, 1923.
RIETZ, H. L., CARVER, H. C., CRATHORNE, A. R., CRUM, W. L., GLOVER, J. W., HUNTINGTON, E.V., KELLEY, T. L., PERSONS, W. M., and YOUNG, A. A. HANDBOOK OF MATHEMATICAL STATISTICS. 221 p., illus. Boston, New York, [etc.] [1924].

The following comparison can then be made:

Method	Standard deviation
Theoretical representative sample, 100 culms	4.95
Series 1, 100 samples, each of 100 culms; 1 culm from each of 100 random places	5.06±0.24
Series 2, 100 samples, each of 100 culms; 20 culms from each of 5 random places	5.73± .28
Series 3, 100 samples, each of 100 culms; 50 culms from each of 2 random places	6.01± .29
Series 4, 100 samples, each of 100 culms; 100 culms all from 1 random place	8.18± .39

Such a range of values would be expected, in that a sample taken all in one place would not be as reliable as one made up of culms selected in several places. The slight difference between the theoretical representative sample and the one taken by the method of one culm from each of 100 places is certainly not significant. Although this method probably has some advantage over the other methods of taking culms in more than one place, the advantage is not great. The variation of these methods from that of taking the culms all in one place, however, is significant, and large enough to deserve consideration.

From actual practice, it has been found that the selection of a sample by taking one culm in a place is much more laborious and demands considerably more time than by taking 20 or 50 in a place. The rather sudden increase in the standard deviation in the case in which all the culms are taken in one place would tend to discourage that method.

Upon study of Formula 2, it will be seen that the standard deviation depends on the percentage of infestation as well as on the number of culms. With a constant number of culms, the standard deviation is at a maximum when $p=q=50$ per cent. With the percentage of infestation constant, the standard deviation varies according to the square root of the number of culms in the sample.

The probable error (PE) of a determination is obtained from the standard deviation by applying the formula

$$PE=0.6745\ \sigma \text{ (Formula 4)}.$$

In the case of a representative sample, $PE=0.6745\times 4.9=3.3$, which means that if a sample of 100 culms taken from a field was found to be 57 per cent infested, the chances would be even that the correct percentage of infestation would lie somewhere within the limitations of 57 ± 3.3. The probable error of the sum or difference of two determinations E_1 and E_2 affected with errors e_1 and e_2 is determined by the formula

$$PE\ (_{E=\ E_2})=\sqrt{e_1^2+e_2^2} \text{ (Formula 5)}.$$

CONCLUSIONS

In general practice in studies of Hessian fly infestation, it is possible to take a sample which quite closely approaches a theoretically representative sample.

In case a representative sample is taken, the probable error is determined by the number of culms taken, and the percentage of infestation.

Whatever the method of taking a sample, the probable error can be computed, and should be recognized and accepted as a part of the determination.

DETERMINATION BY THE 5-SQUARE-YARD METHOD OF THE YIELD OF WHEAT PLOTS USED IN STUDIES OF THE HESSIAN FLY, PHYTOPHAGA DESTRUCTOR SAY [1]

By W. B. CARTWRIGHT, *Assistant Entomologist*, and W. H. LARRIMER, *Entomologist, Cereal and Forage Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

In analyzing the damage of the Hessian fly to wheat, some method of yield-taking seemed necessary whereby the date-of-seeding and the variety plots could be compared. Control experiments supervised by the United States Entomological Field Laboratory, La Fayette, Ind., are scattered from Michigan to Alabama and from West Virginia to Illinois. In order to cope with the difficulties of the harvest period and the routine of cutting, assembling, and threshing, a time- and labor-saving system was chosen. In brief, the method selected consists in harvesting five separated areas, each 1 yard square, which, when taken together, represent 1/968 acre. The spacing of the wheat drill rows makes it possible for each area to consist of five drill rows 1 yard in length. A yardstick, a pair of strong shears or a pocket knife, cloth sacks, and labels constitute the necessary field equipment prior to threshing.

Very little has been published on the limitations of the 5-square-yard method and on the interpretation of records obtained by it. McCall [2] states that preliminary results checked quite satisfactorily with records obtained by threshing entire plots. Arny and Garber [3], in a paper on field technic in determining yields of plots by the rod-row method, add that the removal of at least two border rows from either side of each plot would tend to remove the error of alley defects, which is an objectionable feature of the McCall method. Other inherent errors of yield determination are not fully disclosed, especially the one of random sampling and its significance, which is the subject of the present discussion.

STATISTICAL FORMULAS

Before presenting the results of tests to determine the probable error of the 5-square-yard method, it seems permissible to review several statistical formulas which are referred to later in the text:

FORMULA 1. Arithmetical mean $(M) = \frac{\Sigma fV}{n}$,

where V represents the class magnitude, n the number of sets of observations, f the frequency, and Σ denotes summation.

FORMULA 2. Standard deviation $(\sigma) = \sqrt{\frac{\Sigma fx^2}{n}}$,

[1] Received for publication Nov. 3, 1925; issued June, 1926. This is the second of a series of papers discussing the application of statistical methods to the study of the Hessian fly. The first paper is published in this number of the Journal of Agricultural Research.

[2] MCCALL, A. G. A NEW METHOD FOR HARVESTING SMALL GRAIN AND GRASS PLOTS. Jour. Amer. Soc. Agron. 9: 138-140, illus. 1917.

[3] ARNY, A. C., and GARBER, R. J. FIELD TECHNIC IN DETERMINING YIELDS OF PLOTS OF GRAIN BY THE ROD-ROW METHOD. Jour. Amer. Soc. Agron. 11: 33-47, illus. 1919.

where x represents the deviation of the class from the arithmetical mean.

FORMULA 3. Probable error of a mean $PE_M = 0.6745 \frac{\sigma}{\sqrt{n}}$.

FORMULA 4. Coefficient of variation $(V) = \frac{\sigma}{AM} \times 100$.

FORMULA 5. Probable error of the coefficient of variation

$$(E_V) = 0.6745 \frac{CV}{\sqrt{2n}}.$$

FORMULA 6. Probable error of the sum or difference of two means affected with errors e_1 and $e_2 = \sqrt{e_1^2 + e_2^2}$.

FORMULA 7. Probable error of a general mean of several quantities affected with probable errors $e_1, e_2 \ldots e_n$, $(E_{gm}) = \frac{\sqrt{e_1^2 + e_2^2 \ldots e_n^2}}{n}$, or

$$E_{gm} = \frac{e}{\sqrt{n}}.$$

THE 5-SQUARE-YARD METHOD

As an example of the method and its results, in 1923 a uniform strip of ground was outlined through eight consecutive, yet different, date-of-seeding wheat plots at Centralia, Ill. Twenty-five individual 1-square-yard harvests were made from each plot. The samples were so cut that the 25 values represented five series yields, each of five adjacent square yards. The results of the first of the eight tests are shown in detail in Table I, and the summarized data for all tests in Table II.

TABLE I.—*Distribution of determined bushels of wheat per acre of plot 1, and computation of the standard deviation*

Yield value V	Frequency f	fV	Deviation from average, 14.7 x	x^2	fx^2
Bushels					
21.5	2	43.0	6.8	46.24	92.48
21.0	4	84.0	6.3	39.69	158.76
20.5	9	184.5	5.8	33.64	302.76
20.0	14	280.0	5.3	28.09	393.26
19.5	27	526.5	4.8	23.04	622.08
19.0	36	684.0	4.3	18.49	665.64
18.5	65	1,202.5	3.8	14.44	938.60
18.0	92	1,656.0	3.3	10.89	1,001.88
17.5	125	2,187.5	2.8	7.84	980.00
17.0	152	2,584.0	2.3	5.29	804.08
16.5	192	3,168.0	1.8	3.24	622.08
16.0	222	3,552.0	1.3	1.69	375.18
15.5	249	3,859.5	.8	.64	159.36
15.0	300	4,500.0	.3	.09	27.00
14.5	305	4,422.5	.2	.04	12.20
14.0	296	4,144.0	.7	.49	145.04
13.5	279	3,766.5	1.2	1.44	401.76
13.0	246	3,198.0	1.7	2.89	710.94
12.5	171	2,137.5	2.2	4.84	827.64
12.0	138	1,656.0	2.7	7.29	1,006.02
11.5	99	1,138.5	3.2	10.24	1,013.76
11.0	55	605.0	3.7	13.69	752.95
10.5	29	304.5	4.2	17.64	511.56
10.0	15	150.0	4.7	22.09	331.35
9.5	3	28.5	5.2	27.04	81.12
Totals....	3,125	46,062.5	.....	.....	12,937.50

$$\sigma = \sqrt{\frac{\Sigma fx^2}{n}} = \sqrt{\frac{12,937.5}{3,125}} = 2.03 \text{ bushels}$$

TABLE II.—*Range, average, and dispersion, of yields for plots 1 to 8*

Plot	Range		Average	Standard deviation	Coefficient of variation
	Max.	Min.	*M*	σ	*V*
1	21.5	9.5	14.7	2.03	13.81±.12
2	15.0	8.0	11.3	1.37	12.12±.10
3	23.5	12.5	17.4	1.72	9.88±.08
4	15.0	8.0	11.4	1.43	12.54±.11
5	21.3	13.5	17.2	1.41	8.19±.07
6	22.0	12.0	16.7	1.77	10.59±.09
7	23.5	13.5	19.2	1.70	8.85±.08
8	27.0	18.0	22.4	1.42	6.34±.05

The coefficient of variation introduced in Table II is defined as the percentage of variation in the mean, the standard deviation being treated as the total variation in the mean. Reference is made here to Formula 5 for the computation of its probable error. By Formula 3, since the individual means were derived from one set of observations, n becomes unity, and the probable error of any one mean in the series is $0.6745\ \sigma$, the probable error of a single observation.

As the arithmetical mean of all standard deviations is 1.6, the probable error of the method for practical application can be considered as 1 bushel. From a record of 900 plots, Wood and Stratton[4] found that the probable error of a plot of 1/1,000 acre of mangels was 12 per cent, which approaches the average of the coefficients of variation derived from Table II.

Biometricians have not regarded a difference as significant unless it is at least three times its probable error. A 30-to-1 odd, or 3.8 times the probable error, is of general use. For interpretation, attention is directed to yield values of plots 1 and 8 in Table II. Formula 6 is applied. The difference is 7.7 ± 1.6; the difference divided by its probable error is 4.8, and so would be significant. If a set of observations is repeated, as when investigations are extended over a number of years, the probable error is decreased by the application of Formula 7.

FIVE-SQUARE-YARD AND OTHER SMALL-AREA HARVESTING METHODS COMPARED

Assuming that the 75 feet of drill row comprising the extent of the 5-square-yard method is acceptable, two modified methods were tested, namely, the yard-linear and the 15-foot methods. By the former method the harvest consisted of 25 one-yard lengths of drill row cut in disconnected places in the field; by the latter it consisted of five cuttings of 15 feet each. Incidentally, $\frac{1}{30}$-acre plots were harvested at the same time.

Forty individual tests were made in 1920 with reference to the one-square yard, the yard-linear, and the $\frac{1}{30}$-acre methods. In 1921 and 1922 twenty tests were made on all methods. Data on the three years' work are summarized in Table III.

[4] WOOD, T. B., and STRATTON, F. J. M. THE INTERPRETATION OF EXPERIMENTAL RESULTS. Jour. Agr. Sci. 3: 417–440, illus. 1910.

TABLE III.—*Comparison of harvest methods*

[Average yields per acre]

Year	Method							
	5-square-yard		Yard-linear		15-foot		$\frac{1}{30}$-acre	
	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain
	Pounds	*Bushels*	*Pounds*	*Bushels*	*Pounds*	*Bushels*	*Pounds*	*Bushels*
1920	1,536	13.4	1,275	13.3	.....	.....	947	9.9
1921	2,384	18.8	2,718	20.7	2,589	19.3	1,468	12.6
1922	4,051	28.2	4,193	30.3	3,923	24.7	3,146	21.7

Inasmuch as no corrections could be made for drainage ditches or thinnings in the field in the $\frac{1}{30}$-acre yields, and since there was wastage in shocking, handling, and threshing not common to the smaller harvests made at the same time, it is not unreasonable to expect the lower yield values shown in Table III. The difference noted in the other yields would not be significant, owing to the probable error of the methods, so the inferences are that no greater accuracy could be assured by adopting either the yard-linear or the 15-foot method in preference to the 5-square-yard standard.

SUMMARY

Field harvests by the 5-square-yard method showed an error of sampling ranging from 6.3 to 13.8 per cent, and an average error of 10.3 per cent, of the computed average yields. Other $\frac{1}{968}$-acre harvests did not prove superior in accuracy or in refinement of determination over the 5-square-yard method.

DETERMINATION OF THE PERCENTAGE OF PARASITISM OF THE HESSIAN FLY, PHYTOPHAGA DESTRUCTOR SAY [1]

By W. H. LARRIMER, *Entomologist*, and W. B. NOBLE, *Junior Entomologist*, *Cereal and Forage Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

Severe outbreaks of the Hessian fly have occurred at more or less regular intervals of time since its introduction into this country, supposedly during the Revolutionary War. At present more than 20 species of Hymenoptera commonly occur as parasites of this pest, and in some cases at least the decline in severity of an outbreak has been accompanied by an increase in the percentage of parasitism by one or many of these species. Although there can be little doubt that its natural insect enemies play an important part in reducing a severe outbreak of the Hessian fly, adverse climatic conditions, and in recent years the intentional manipulation of its favorite food plants so that they may escape infestation, are also factors, which, it is thought, deserve more credit in this respect than has hitherto been given them.

THE PROBLEM

Preliminary to an attempt to study the various relations of host and parasites it became advisable to select a method of determination of percentage of parasitism by which a comparative study could be made of the parasitism occurring under various conditions. The following discussion deals with this particular phase of the problem.

DISCUSSION

It would seem that the only way to be certain of the exact percentage of parasitism of the "flaxseeds" occurring in a certain field would be to examine every one in that field and make no mistakes. Such a procedure can be considered humanly impossible, but it must be admitted that any other is subject to error. The other extreme would be to examine one flaxseed and take the result as an indication of either 100 per cent or no parasitism. The error of this method is obviously too great. The question then arises as to what would be the minimum number of flaxseeds to select and examine in order to obtain a percentage of parasitism near enough to the exact percentage to be of practical value for the purpose desired.

In actual practice, the source of flaxseeds to be examined can not always be selected as desired, but in most cases will depend on circumstances which make a suitable number of flaxseeds available. Until the field habits of the parasites involved are much more completely

[1] Received for publication Nov. 3, 1925; issued June, 1926. This is the third of a series of papers discussing the application of statistical methods to the study of the Hessian fly. The first and second are published in this number of the Journal of Agricultural Research.

known, any method of obtaining what is considered a fairly representative lot of flaxseeds can be used.

In order to get some figures on which to base an opinion, flaxseeds were obtained from culms taken at random in lots of from 25 to 50 in several different locations over a field. All flaxseeds were removed from all of the culms and thoroughly mixed together. The first five flaxseeds containing, or that had contained, either the fly or parasitic forms were included in one group, the first group of five; and the next five dissected made up the second group of five; and so on. Flaxseeds from which flies or their parasites had emerged were included, but flaxseeds containing dead forms were excluded. A number of factors which theoretically, and no doubt actually, are causes of error were carefully considered, but on the grounds of practical application were excluded although not entirely disregarded.

Three hundred flaxseeds, exclusive of those dead, were thus selected and dissected, and the results were recorded by groups of five. The five-group was selected as being small enough to show suitable gradation, as well as being easily combined into larger groups of convenient size. The first group of 10 was made by combining the first and second groups of five; the first group of 15, by combining the first, second, and third groups of five, and so on. From results thus obtained, showing percentages of parasitism of groups containing numbers of flaxseeds in multiples of five from 5 to 50, considerable variation was observed.

The standard deviation is more or less commonly used as a measure of the dispersion of a set of observations around their arithmetical mean. There is a theoretical formula for the determination of the standard deviation in such cases:

$$\text{Standard deviation, } \sigma, = \sqrt{\frac{pq}{n}},$$

where p is the percentage of flaxseeds parasitized, q the percentage of flaxseeds not parasitized, and n the number of flaxseeds examined for each percentage determination. By this formula it is possible to determine theoretically the standard deviations of the groups, with no tedious collections, dissections, or, indeed, complicated calculations. The standard deviations of the groups were determined by this formula, and are given in the third column of Table I.

TABLE I.—*Groups of from 5 to 50 with the number of percentage determinations for each group and the respective theoretical and empirical standard deviations*

Group	n	Standard deviation	
		Theoretical	Empirical
5	60	16.4	17.2±1.1
10	30	11.6	13.6±1.2
15	20	9.5	9.8±1.1
20	15	8.2	9.6±1.2
25	12	7.3	7.9±1.1
30	10	6.7	6.9±1.1
35	8	6.2	5.7±1.0
40	7	5.8	5.2± .9
45	6	5.5	4.6± .9
50	6	5.2	6.3±1.2

Although the development of this formula had been followed a number of times, a more or less practical test was necessary before entire confidence could be placed in it. Standard deviation may be defined as the square root of the mean of the squares of the deviations from the arithmetical mean. By reference to this definition, it will be seen that the standard deviation can be determined experimentally according to the formula

$$\text{Standard deviation, } \sigma, = \sqrt{\frac{\Sigma f x^2}{n}},$$

where x is the deviation from the mean, f the frequency of occurrence of like deviations, n the number of percentage determinations in each group, and Σ denotes summation. The standard deviations for the groups of 5 to 50 were determined accordingly.

These empirical or experimental determinations are given in the fourth column of Table I, where they can easily be compared with the corresponding theoretical determinations. It is necessary, however, to obtain the probable error of the experimentally determined standard deviations before an intelligent comparison can be made. This can be done by the formula

$$PE\,\sigma = \frac{0.6745\,\sigma}{\sqrt{2n}},$$

where σ is standard deviation, and n the number of percentage determinations of the group.

The number of percentage determinations in each group is comparatively small, and the results can therefore be taken only as indicative. There is no significant difference, however, between the standard deviations determined theoretically and those determined by experience.

As the probable error of a determination is used to express the dispersion of a set of observations more often than the standard deviation, their respective relation may be expressed

$$PE\,\sigma = 0.6745\,\sigma.$$

It will be seen that the theoretical standard deviation and probable error are dependent on the number of flaxseeds examined and the percentage of parasitism. Thus the probable error can be reduced by increasing the size of the sample; however, if it is necessary for any reason to limit the number of flaxseeds to be examined, the corresponding probable error of the resultant determination must be accepted.

CONCLUSION

Considerable confidence can be placed on a percentage of parasitism of the Hessian fly expressed by the percentage of parasitism of a sample lot of flaxseeds with the appropriate probable error determined theoretically.

A TWO-YEAR STUDY OF THE DEVELOPMENT OF THE EUROPEAN CORN BORER IN THE NEW ENGLAND AREA[1]

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INTRODUCTION

Knowledge of the life history of an insect is often based on insectary rearings supplemented by a few field observations. It is a question whether such information is to be relied on in charting seasonal occurrence from which recommendations for control may be drawn.

In the studies of the European corn borer in the New England area this question was raised several years ago, and attention was directed to the field in order to learn, if possible, just what the seasonal occurrence of the insect was under natural conditions. During 1922 and 1923 this subject was studied by a system of field counts. Since the insect in the larval stage is found on many hosts, the counts included not only corn but several other preferred food plants, and were taken in numerous localities so that the whole area might be fairly represented. Counts of 100 individuals each were made throughout the periods of pupation and emergence in each generation each year, this figure being chosen because of the ease with which such units could be reduced to a percentage basis. The examinations were mainly of pupation and emergence, because a knowledge of the development of these phenomena forms a basis from which, knowing the details of life history, seasonal charts can be constructed with a fair degree of accuracy. The same degree of accuracy can not be obtained in percentage counts of eggs and young larvae.

EXPERIMENTAL DATA

Pupation and emergence records for each generation in 1922 and 1923 are shown in Tables I and II. In these tables the daily records are in percentage of development of stages, and are often averages for several counts made on one date. Thus in the case of the columns headed "Total corn," the percentage figure given on a particular date was usually derived from several counts of 100 individuals each observed in corn in several localities. Such a figure represents the condition of the area as a whole in as fair a manner as is possible. For some hosts listed in Tables I and II fewer records are given than for others. For example, in Table I under "1923—Overwintering generation," fewer counts are shown in "Revere corn" than in "Arlington corn." Such a condition exists simply because more insects were found in certain localities or in certain food plants than in other localities or in other food plants, thus making possible a larger number of counts of individuals in one case than in another.

[1] Received for publication November 3, 1925; issued June, 1926.

TABLE I.—*Field records of pupation and emergence of the overwintering generation of the European corn borer in New England April 18 to June 28, 1922 and 1923*

Date	1922. Overwintering generation						1923. Overwintering generation																											
	Pupation [a]			Emergence [b]			Pupation [a]									Emergence [b]																		
	Total corn	Cocklebur	Barnyard grass	Total corn	Cocklebur	Barnyard grass	Total corn	Total weeds	Total cocklebur	Total barnyard grass	Total Amaranthus	Arlington corn	Arlington barnyard grass	Arlington Amaranthus	Revere corn	Revere Amaranthus	Melrose corn	Total corn	Total weeds	Total cocklebur	Total barnyard grass	Total Amaranthus	Arlington corn	Arlington barnyard grass	Arlington Amaranthus	Revere corn	Revere Amaranthus	Melrose corn						
Apr. 18	.....	0.03	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
22	.....	.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
24	.....	.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
25	.....	.4	1.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
26	.....	.4	.....	.....	.....	.....	.....	1.1	.....	1.0	.....	.....	1.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
27	0.8	.....	.9	.....	.....	.....	.....	1.8	1.1	.....	2.1	.....	.....	2.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
28	1.8	2.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
29	10.7	4.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
30	.....	.....	.....	.....	.....	.....	3.1	1.3	.9	4.5	.....	3.1	4.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
May 1	1.1	6.5	.....	.....	.....	.....	1.1	2.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
2	.....	.....	.....	.....	.....	.....	.5	4.3	.....	4.1	4.5	.....	4.1	4.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
3	16.7	14.1	.....	.....	.....	.....	1.8	3.1	3.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
4	15.9	.....	.....	.....	.....	.....	4.3	2.1	.....	.....	4.2	4.3	.....	4.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
5	.....	.....	.....	.....	.....	.....	4.0	10.0	.....	9.0	11.0	4.0	9.0	11.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
6	13.3	14.0	22.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
7	.....	.....	.....	.....	.....	.....	4.3	15.6	18.0	16.0	13.0	8.0	16.0	13.0	4.0	.....	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
8	13.8	.....	33.3	.....	.....	.....	6.5	31.4	30.0	32.0	26.0	13.0	32.0	26.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
9	25.0	16.0	.....	.....	.....	.....	9.3	27.5	.....	8.0	34.0	14.0	8.0	41.0	8.0	20.0	6.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
10	18.1	.....	.....	.....	.....	.....	27.0	44.0	.....	39.0	49.0	27.0	39.0	49.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
11	31.7	.....	.....	.....	.....	.....	21.0	28.7	.....	20.5	35.0	29.0	39.0	37.0	8.0	40.0	18.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
12	21.5	37.4	31.4	.....	.....	.....	28.0	42.0	.....	35.0	47.0	38.0	35.0	49.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
13	41.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
14	.....	.....	.....	.....	.....	.....	29.7	45.0	.....	44.0	44.0	30.0	44.0	43.0	15.0	48.0	24.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
15	68.7	.....	80.0	.....	.....	.....	29.0	37.3	30.0	46.0	49.0	36.0	46.0	49.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
16	52.9	50.8	65.4	.....	.....	0.7	19.5	44.9	49.0	54.0	58.0	14.5	54.0	58.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
17	68.3	59.6	.....	.....	.....	.....	22.0	45.0	26.0	46.0	54.0	30.0	46.0	56.0	22.0	52.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
18	.....	.....	.....	.....	.....	.....	49.7	63.0	61.0	63.0	69.0	52.0	63.0	69.0	.....	.....	33.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
19	79.4	59.1	62.7	0.9	2.0	1.5	37.7	64.7	.....	69.0	71.0	34.0	69.0	70.0	48.0	73.0	.....	.....	.....	.....	.....	.....	1	.....	.....	1	.....	.....						
20	71.2	63.0	89.6	.....	2.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						
21	.....	.....	.....	.....	.....	.....	44.0	73.0	.....	.....	73.0	73.0	.....	73.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....						

	22		71.8	68.9		1.1	10.3		67.4	77.6	83.0	76.0	84.0	70.0	76.0	84.0	57.0	86.0	52.0										1					
	23		80.2	63.4		2.9	7.2		59.7	72.2	47.0	84.0	80.0	52.0	84.0	80.0					0.2		1			1									
	24		61.3	72.2		1.2	7.8		57.8	64.1		64.0	82.0	58.0	76.0	81.0	58.0	86.0	56.0	0.1	1.4		2			4	2.0	1	1						
	25		89.2	84.3	89.6	11.0	11.7	3.4	68.6	76.0		76.0	94.0	72.0	76.0	94.0				.6	.3		1		2	1									
	26		88.4	92.6		7.7	30.4		74.4	91.7	58.0	92.0	95.5	82.0	92.0	98.0	71.0	93.0	48.0	.4	1.0		2	1.0	1	2			2					
	27			93.9	94.9		20.7	11.6																											
	28								84.7	96.8	72.0	88.0	92.0	81.0	88.0	94.0	91.0	90.0	79.0	2.0	1.3	2.0	4	.5	2	4			1						
	29			87.6			26.2		86.0	87.4	83.0	89.0	94.0	89.7	89.0	94.0			79.0	1.7	5.0	3.5	4	13.0	2	4	13.0			1.0					
	31		100.0	93.2		23.7	40.1		85.8	92.3	89.0	94.0	98.0	89.0	88.0	100.0	87.5	96.0	74.0	3.3	10.1	7.5	2	17.0	5	2	18.0	3	16					
June	1		97.7	97.5		25.4	43.8		89.6	96.0		94.0	98.0	88.0	94.0	98.0				10.0	14.5		8	21.0	6	8	21.0								
	2		98.1	97.6	98.0	36.6	35.4	46.0	91.6	91.2	90.0	98.0	94.0	97.0	88.0	94.0	89.0	94.0	92.0	10.2	25.4	34.0	10	29.5	13	10	30.0	2	29	1.0					
	3			100.0			52.4																												
	4								90.4	94.1	94.0	94.0	98.0	96.0	94.0	98.0	95.0		95.0	10.2	48.0	43.0	36	70.0	20	36	70.0	17		3.0					
	5		99.6	99.0		62.9	57.6		96.0	96.6	97.0	98.0	95.0	93.0	98.0	95.0				35.5	44.6	44.0	45	45.0	27	45	45.0								
	6		98.5	100.0		59.0	69.0		95.3	98.8		97.0	100.0	97.0	97.0	100.0		100.0		19.7	58.8		55	61.4	23	55	64.0		56					
	7		100.0	99.0		62.0	80.4		95.6	95.4	93.0	98.0	92.0	90.0	98.0	92.0				39.0	62.8	57.0	61	78.0	43	61	78.0								
	8		98.6	99.7		89.7	88.5		86.8											15.8														
	9		100.0	98.7		81.7	75.0																												
	10			97.0			82.7																												
	11								97.5	98.0		99.0	97.0	95.0	99.0	97.0	100.0		97.0	58.4	71.0		77	65.0	52	77	65.0	56		39.0					
	12		100.0	99.6		89.6	94.5		96.6	92.5	90.0	99.0	98.0	92.0	99.0	98.0			98.0	48.5	66.5	61.0	72	78.0	55	72	78.0			45.0					
	13		100.0	100.0		85.9	95.0		97.3	97.7	96.6	100.0	98.0	94.0	100.0	98.0	99.0		99.0	50.4	69.7	63.0	80	86.0	60	80	86.0	65		35.0					
	14		99.5	100.0		94.1	95.8		99.7	97.6	95.5	100.0	98.5	100.0	100.0	98.5			99.0	70.6	80.6	71.5	79	90.5	82	79	90.5			46.0					
	15		98.9			87.9			99.0	100.0		100.0	100.0		100.0	100.0	97.0		98.0	67.6	84.3		90	93.0		90	93.0	71		61.0					
	16		100.0		100.0	95.0		89.4	100.0	99.5		99.0	100.0		99.0	100.0				88.0	84.5		77	92.0		77	92.0								
	18								99.0	100.0		100.0	100.0		100.0	100.0			98.0	86.0	92.0		88	96.0		88	96.0			71.0					
	19								100.0	99.5		99.0	100.0		99.0	100.0	100.0		100.0	88.5	94.5		94	95.0		94	95.0	95		82.0					
	20			100.0			99.0		100.0	100.0	100.0	100.0	100.0		100.0	100.0			100.0	77.0	93.3	90.0	95	95.0		95	95.0			77.0					
	21								100.0	100.0		100.0	100.0		100.0	100.0	100.0		100.0	94.0	97.5		98	97.0		98	97.0	94		92.0					
	22		100.0			100.0			100.0	98.7	98.0	99.0	99.0		99.0	100.0			100.0	95.0	92.1	92.0	89	96.0		89	96.0			95.0					
	23		100.0			100.0																													
	25								99.7	100.0		100.0	100.0		100.0		100.0		99.3	93.3	99.5		99	100.0		99	100.0	94		97.3					
	26								100.0	100.0	100.0	100.0			100.0				100.0	97.0	99.3	98.0		100.0		100	100.0			97.0					
	27								100.0								100.0		100.0	98.7								100		96.0					
	28								100.0										100.0	99.0										99.0					

• Percentage of total larvæ that had pupated on a given date.

♭ Percentage of empty pupal shells.

TABLE II.—*Field records of pupation and emergence of the first generation of the European corn borer in New England July 10 to September 13, 1922 and 1923*

Date	1922. First generation								1923. First generation							
	Pupation [a]				Emergence [b]				Pupation [a]				Emergence [b]			
	Total corn	Arlington corn	Saugus corn	Cocklebur	Total corn	Arlington corn	Saugus corn	Cocklebur	Total corn	Melrose corn	Medford corn	Dock	Total corn	Melrose corn	Medford corn	Dock
July 10									[c]							
12	0.9		0.9									3				
13	.2			2.5												
14				9.0												
15	7.7			3.3												
17	6.0		6.0	11.0					0.4		2.0	4				
18	13.7		12.0	13.0					.3		1.0	6				
19	8.3		8.3	28.0					1.1		5.0	8	[c]		[c]	
20	26.0								1.0		3.0					
21	6.2	8.1	16.3	38.0					8.0		16.0					
22	18.0															
23									9.0		18.0	23	[c]		[c]	
24	28.7	15.0	28.8	47.0	0.1		0.2		4.0	1	11.0					
25	52.5			53.0	1.0											
26	17.8	19.0	43.0	34.9					10.7		9.0	46				
27	43.0		52.6		.6		1.2		16.4		19.0		0.4			
28	57.3	19.0	38.0	53.0	3.7	1.0		4.0				31				
29	58.8		58.8	67.6	.3		.3									
30									28.0				1.0			
31	76.4		65.8	72.0	7.9		2.5	4.0	35.0			61	2.0			1
Aug. 1	54.2	72.7		75.0	3.0	3.0		5.0	2.5	5		66				2
2	68.0		68.0	73.0				6.0	10.2	6	42.0					
3	64.3		82.2		8.8		15.6		24.6	8	54.0	72	.8		2	3
4	92.2	84.5	82.0	78.0	34.0	9.0	11.0	17.0	25.0	25						
5	100.0				38.6											
6									45.7	43	72.0	87	.7		3	10
7	93.7		83.7	69.0	50.5		4.1	16.0								
8	91.0		86.1	82.0	47.5		34.4	13.0	75.0		75.0	90	3.0		3	10
9	81.7	83.5	86.7		21.2	18.5	41.2		62.0	65	76.0	90	2.7		8	19
10	88.2	81.7	89.2	74.0	37.0	27.7	37.4	19.4								
11	88.2		87.7	90.5	38.6		34.3	55.0	59.7	61	73.0		5.3	1	15	
12	97.6				75.6											
13									71.0	78	91.0	98	8.0	4	34	52
14	80.0	90.0	88.0	87.0	46.4	60.0	43.0	55.0	91.0	94	88.0	99	18.0	4	32	69

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In considering Tables I and II the reader may more readily understand the way in which the figures were obtained and what they represent if one day's record is explained. In Table I, May 26 may be chosen for the purpose of illustration. Under the heading "Total corn," pupation was found to be 88.4 per cent and emergence 7.7 per cent. These are average figures for several counts of 100 individuals each, made in corn, in different localities. By May 26, therefore, the total percentage of larvae that had pupated in corn was 88.4, and the counts showed an average of 7.7 per cent of cast skins, that is, pupae from which adult insects had issued, or briefly, emergence. The figure 88.4 per cent given as pupation therefore includes also those individuals that had passed beyond the pupal stage. It was found, then, that on May 26, 1922, there was in corn in eastern New England an average of 11.6 per cent of overwintering larvae, 80.7 per cent of pupae, and 7.7 per cent of adults. With this explanation in mind the advancement of pupation or emergence of the insect on a certain date may be noticed in several foods plants and in various localities for each generation during the seasons 1922 and 1923.

It may be noticed in Tables I and II that considerable variation in development of the insect in corn is shown. In Table I, for example, under the heading "Pupation, Total corn," pupation of 25 per cent is recorded on May 9, whereas on May 10 the records show 18.1 per cent. These records might seem to be contradictory if not fully understood. If it is desired to obtain records of development for 40 fields of corn as representing a certain area and it is possible to examine only 10 of these fields on a given date, a record of development in each individual field might be obtained once every four days. In case the progress of development was not exactly the same in each field, the daily averages would show such apparent inconsistencies as are indicated in Table I. When these figures are smoothed into a curve of development, however, such inconsistencies for the most part disappear. The same condition is observed in making daily counts of development in one field, although in this case the variation in the daily figures is usually found to be less marked. In each case it is necessary to smooth the figures to obtain a curve of development of the desired regularity.

It may be seen from these records that considerable variation appeared in development as between two localities, two food plants, or the two years. When smoothed curves are constructed and compared, this variation becomes clearer.

In Tables III and IV this difference is shown as average daily percentage of development. Thus in Table III, first item, it was found that pupation in corn advanced on an average 1.4 per cent faster than in cocklebur in 1922. This means that on each day during the pupation of the insect in the field the pupation was on an average 1.4 per cent greater in corn than in cocklebur. The average figure was obtained by averaging the daily difference between the smoothed curves for the whole period during which pupation occurred in the field. A few discrepancies shown in these tables were caused by the necessary correcting of a few smoothed curves by hand.

TABLE III.—*Variation in the development of the European corn borer expressed in average percentage of difference*

1922. SECOND GENERATION (OVERWINTERING)

Pupation in corn advanced on an average 1.4 per cent faster than in cocklebur.
Pupation in barnyard grass advanced on an average 6.8 per cent faster than in corn.
Pupation in barnyard grass advanced on an average 7.8 per cent faster than in cocklebur.

1922. FIRST GENERATION

Pupation in cocklebur advanced on an average 6.4 per cent faster than in corn.
Pupation in Saugus corn advanced on an average 9 per cent faster than in Arlington corn.

1923. SECOND GENERATION (OVERWINTERING)

Pupation in weeds advanced on an average 7.7 per cent faster than in corn.
Pupation in Amaranthus advanced on an average 9.4 per cent faster than in corn.
Pupation in cocklebur advanced on an average 2.5 per cent faster than in corn.
Pupation in barnyard grass advanced on an average 9.1 per cent faster than in corn.
Pupation in Arlington corn advanced on an average 4.6 per cent faster than in Revere corn.
Pupation in Arlington corn advanced on an average 8.2 per cent faster than in Melrose corn.
Pupation in Arlington Amaranthus advanced on an average 9.5 per cent faster than in Arlington corn.
Pupation in Arlington barnyard grass advanced on an average 6.8 per cent faster than in Arlington corn.
Pupation in Arlington Amaranthus advanced on an average 1.5 per cent faster than in Arlington barnyard grass.
Pupation in Arlington Amaranthus advanced on an average 0.07 per cent faster than in Revere Amaranthus.
Pupation in Revere Amaranthus advanced on an average 19.6 per cent faster than in Revere corn.

1923. FIRST GENERATION

Pupation in dock advanced on an average 27.3 per cent faster than in corn.
Pupation in Medford corn advanced on an average 23.3 per cent faster than in Melrose corn.

1922 VERSUS 1923. SECOND GENERATION

Pupation in 1922 corn advanced on an average 13 per cent faster than in 1923 corn.
Pupation in 1922 cocklebur advanced on an average 6.8 per cent faster than in 1923 cocklebur.
Pupation in 1922 barnyard grass advanced on an average 7.6 per cent faster than in 1923 barnyard grass.

1922 VERSUS 1923. FIRST GENERATION

Pupation in 1922 corn advanced on an average 28.4 per cent faster than in 1923 corn.

TABLE IV.—*Variation in the development of the European corn borer expressed in average percentage of difference*

1922. SECOND GENERATION (OVERWINTERING)

Emergence in cocklebur advanced on an average 7.3 per cent faster than in corn.
Emergence in corn advanced on an average 0.4 per cent faster than in barnyard grass.
Emergence in cocklebur advanced on an average 8.1 per cent faster than in barnyard grass.

1922. FIRST GENERATION

Emergence in corn advanced on an average 1.9 per cent faster than in cocklebur.
Emergence in Arlington corn advanced on an average 1.8 per cent faster than in Saugus corn.

1923. SECOND GENERATION (OVERWINTERING)

Emergence in weeds advanced on an average 10.9 per cent faster than in corn.
Emergence in Amaranthus advanced on an average 15.5 per cent faster than in corn.
Emergence in cocklebur advanced on an average 6.5 per cent faster than in corn.
Emergence in barnyard grass advanced on an average 9.6 per cent faster than in corn.
Emergence in Arlington corn advanced on an average 1.4 per cent faster than in Revere corn.
Emergence in Arlington corn advanced on an average 16.3 per cent faster than in Melrose corn.
Emergence in Arlington Amaranthus advanced on an average 19.6 per cent faster than in Arlington corn.
Emergence in Arlington barnyard grass advanced on an average 8.7 per cent faster than in Arlington corn.
Emergence in Arlington Amaranthus advanced on an average 5.7 per cent faster than in Arlington barnyard grass.
Emergence in Arlington Amaranthus advanced on an average 2.4 per cent faster than in Revere Amaranthus.
Emergence in Revere Amaranthus advanced on an average 13.6 per cent faster than in Revere corn.

1923. FIRST GENERATION

Emergence in dock advanced on an average 27.6 per cent faster than in corn.
Emergence in Medford corn advanced on an average 28.6 per cent faster than in Melrose corn.

1922 VERSUS 1923. SECOND GENERATION

Emergence in 1922 corn advanced on an average 20.5 per cent faster than in 1923 corn.
Emergence in 1922 cocklebur advanced on an average 18.1 per cent faster than in 1923 cocklebur.
Emergence in 1922 barnyard grass advanced on an average 12.7 per cent faster than in 1923 barnyard grass.

1922 VERSUS 1923. FIRST GENERATION

Emergence in 1922 corn advanced on an average 25 per cent faster than in 1923 corn.

This variation of development may also be shown by average difference in days in progress of development, as in Tables V and VI. Thus in Table V pupation in corn was on an average 0.6 days more advanced than in cocklebur. This average was obtained by averaging difference in days throughout the period of pupation.

TABLE V.—*Variation in the development of the European corn borer expressed in average days of difference*

1922. SECOND GENERATION (OVERWINTERING)

Pupation in corn was on an average 0.6 days more advanced than in cocklebur.
Pupation in barnyard grass was on an average 2.7 days more advanced than in corn.
Pupation in barnyard grass was on an average 3 days more advanced than in cocklebur.

1922. FIRST GENERATION

Pupation in cocklebur was on an average 1.3 days more advanced than in corn.
Pupation in Saugus corn was on an average 3.1 days more advanced than in Arlington corn.

1923. SECOND GENERATION (OVERWINTERING)

Pupation in weeds was on an average 3.8 days more advanced than in corn.
Pupation in Amaranthus was on an average 5.3 days more advanced than in corn.
Pupation in cocklebur was on an average 1.3 days more advanced than in corn.
Pupation in barnyard grass was on an average 4.4 days more advanced than in corn.
Pupation in Arlington corn was on an average 2.2 days more advanced than in Revere corn.
Pupation in Arlington corn was on an average 3.5 days more advanced than in Melrose corn.
Pupation in Arlington Amaranthus was on an average 4.3 days more advanced than in Arlington corn.
Pupation in Arlington barnyard grass was on an average 3.3 days more advanced than in Arlington corn.
Pupation in Arlington Amaranthus was on an average 0.8 days more advanced than in Arlington barnyard grass.
Pupation in Arlington Amaranthus was on an average 0.5 days more advanced than in Revere Amaranthus.
Pupation in Revere Amaranthus was on an average 7.2 days more advanced than in Revere corn.

1923. FIRST GENERATION

Pupation in dock was on an average 8.7 days more advanced than in corn.
Pupation in Medford corn was on an average 7 days more advanced than in Melrose corn.

1922 VERSUS 1923. SECOND GENERATION

Pupation in 1922 corn was on an average 5.3 days more advanced than in 1923 corn.
Pupation in 1922 cockebur was on an average 2.2 days more advanced than in 1923 cocklebur.
Pupation in 1922 barnyard grass was on an average 3 days more advanced than in 1923 barnyard grass.

1922 VERSUS 1923. FIRST GENERATION

Pupation in 1922 corn was on an average 9.4 days more advanced than in 1923 corn.

TABLE VI.—*Variation in the development of the European corn borer expressed in average days of difference*

1922. SECOND GENERATION (OVERWINTERING)

Emergence in cocklebur was on an average 2.4 days more advanced than in corn.
Emergence in corn was on an average 0.01 days more advanced than in barnyard grass.
Emergence in cocklebur was on an average 2.5 days more advanced than in barnyard grass.

1922. FIRST GENERATION

Emergence in cocklebur was on an average 0.1 days more advanced than in corn.
Emergence in Arlington corn was on an average 0.8 days more advanced than in Saugus corn.

1923. SECOND GENERATION (OVERWINTERING)

Emergence in weeds was on an average 3.7 days more advanced than in corn.
Emergence in Amaranthus was on an average 4.9 days more advanced than in corn.
Emergence in cocklebur was on an average 1.9 days more advanced than in corn.
Emergence in barnyard grass was on an average 3.3 days more advanced than in corn.
Emergence in Arlington corn was on an average 0.4 days more advanced than in Revere corn.
Emergence in Arlington corn was on an average 4.3 days more advanced than in Melrose corn.
Emergence in Arlington Amaranthus was on an average 5 days more advanced than in Arlington corn.
Emergence in Arlington barnyard grass was on an average 2.3 days more advanced than in Arlington corn.
Emergence in Arlington Amaranthus was on an average 1.9 days more advanced than in Arlington barnyard grass.
Emergence in Arlington Amaranthus was on an average 0.6 days more advanced than in Revere Amaranthus.
Emergence n Revere Amaranthus was on an average 4.5 days more advanced than in Revere corn.

TABLE VI.—*Variation in the development of the European corn borer expressed in average days of difference*—Continued

1923. FIRST GENERATION

Emergence in dock was on an average 8.4 days more advanced than in corn.
Emergence in Medford corn was on an average 9.2 days more advanced than in Melrose corn.

1922 VERSUS 1923. SECOND GENERATION

Emergence in 1922 corn was on an average 6.4 days more advanced than in 1923 corn.
Emergence in 1922 cocklebur was on an average 6.4 days more advanced than in 1923 cocklebur.
Emergence in 1922 barnyard grass was on an average 3.6 days more advanced than in 1923 barnyard grass.

1922 VERSUS 1923. FIRST GENERATION

Emergence in 1922 corn was on an average 11.8 days more advanced than in 1923 corn.

REASONS FOR DIFFERENCES IN DEVELOPMENT

Not all of the reasons for these differences in development are well understood, but the following may be offered as a partial explanation of certain of those noticed.

THE ADVANCEMENT OF THE SEASON.—The spring phenomena, such as the appearing of leaves on trees, increase in warmth, etc., were from 10 to 14 days later in 1923 than in 1922. The effect of this on the development of the corn borer is shown in the comparisons under the heading "1922 versus 1923. Second generation" in Tables III to VI.

SEASONAL TEMPERATURE.—Warm weather prevailed during the transformation of the first generation in 1922, but in 1923 the weather was considerably colder. The possible effect of this condition is evident in the difference shown in pupation and emergence in Tables III to VI under the heading "1922 versus 1923. First generation."

TIME OF PLANTING.—The time of planting corn also affects the development of the first-generation pupae and adults. Thus corn planted 10 days earlier may receive eggs several days sooner, and if development of the insect progresses with the same rapidity in each field the larvae will become full-grown and transform sooner than in corn planted later. Possible instances of this are shown in comparisons of Saugus and Arlington corn in Tables III to VI under the heading "1922. First generation" and of Medford and Melrose corn under the heading "1923. First generation."

HOST PLANT.—Development of pupae and adults in weeds is usually considerably more advanced than in corn on the same date. This is shown in several instances in Tables III, IV, V, and VI. Several factors may operate to bring about this condition, one of which is the fact that smaller stalks such as barnyard grass (*Echinochloa* sp.), pigweed (*Amaranthus* sp.), and cocklebur (*Xanthium* sp.), dry out and warm up in the spring sooner than corn, which has a relatively larger stalk, retains winter moisture longer, and is less directly warmed by the sun's rays in the spring.

THE CASE OF DOCK.—Dock appears quite early in the spring, and is well matured by the time the summer brood of adults appears. The first records of eggs have usually been obtained on it, the large succulent rib of the leaves furnishing ideal feeding quarters for the young larvae before the main stalk has developed. This plant, therefore, obtains the very earliest eggs deposited, even before corn has appeared above the ground. This, in part, accounts for the more rapid devel

opment in dock shown in Tables III to VI under the heading "1923. First generation."

The progress of pupation and emergence also varies considerably with the condition of the host plant. Thus in corn, development is considerably more rapid in stubble and in stalks lying on the ground than in standing stalks. An illustration of such difference in development of larvae in stubble and standing stalks is shown in Table VII. This no doubt is caused by the fact that the soil absorbs heat more rapidly than standing stalks, and transmits it to stubble and fallen stalks.

TABLE VII.—*Comparison of the development of the European corn borer in standing corn and in corn stubble, in Melrose, Mass., in 1923*

Date on which counts were made	Standing corn			Corn stubble		
	Larvae	Pupae	Emerged	Larvae	Pupae	Emerged
May 11	82	18	0	60	40	0
May 14	76	24	0	50	50	0
May 18	67	33	0	28	72	0
May 26	52	48	0	11	89	0
May 29	21	78	1	6	90	4
June 2	8	91	1	3	73	24
June 11	3	58	39	1	21	78
June 14	1	53	46	0	24	76
June 18	2	27	71	0	0	100

Having at hand accurate data of pupation and emergence of the insect in the field, the construction of a seasonal chart is possible only by combining this information with knowledge of the life history as obtained under cage conditions. The life-history data as obtained for 1922 are as follows:

Length of life of adults	First generation of 1922 average	15.5 days.
	Adults of overwintering larvae from 1921 average	15.9 days.
Preoviposition period	First generation of 1922 average	5.0 days.
	Adults of overwintering larvae from 1921 average	4.9 days.
Oviposition period	First generation of 1922 average	7.2 days.
	Adults of overwintering larvae from 1921 average	6.8 days.
Incubation period	First generation of 1922 average	7.4 days.
	Adults of overwintering larvae from 1921 average	5.9 days.

Figure 1 shows the seasonal occurrence of the European corn borer in the New England area in 1922. It takes into account development in all localities and in all food plants examined in 1922 and is an attempt to present as accurately as possible the seasonal occurrence of the insect in the area as a whole.

EXPLANATION OF CHARTS

The following explanation of the plan followed in the construction of the charts is offered:

OVERWINTERING LARVAE.—The chart of this stage is shown as beginning April 15, a few days before the first record of pupation in the field. This date was selected purely for convenience, for the

chart might have been shown as extending from the previous fall through the winter. On April 15 it shows 100 per cent of larvae, since no pupation had been found. The curve of decrease of larvae begins with the discovery of the first pupa on April 18 (Table I), and from that date the curve of decrease continues to show a smaller percentage of larvae in the field each day until the last larva pupated, or until larvae were no longer found in the field counts.

It has been previously stated that Figure 1 shows the development of the insect in all localities and on all known host plants in the New England area. The larvae of this insect entered hibernation in the

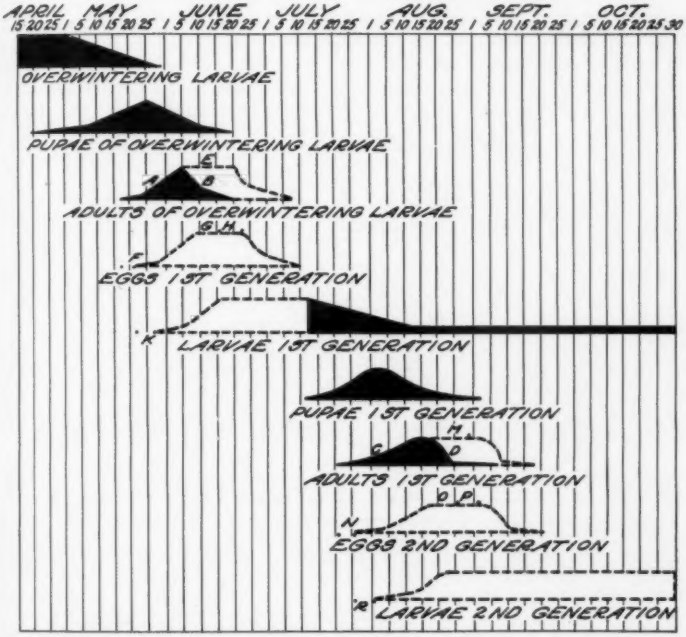

FIG. 1.—Seasonal occurrence of the European corn borer, in the field, in the New England area, during 1922. The solid black areas were constructed from field records. The white areas were computed from information obtained in life-history studies

fall of 1921 in cornstalks, and in many other species of plants, particularly weeds. In the infested area as a whole, a large percentage of these larvae were in cornstalks and the majority of the remainder were in favored host plants such as cocklebur (*Xanthium* sp.) and barnyard grass (*Echinochloa crusgalli* L. (Beauv.)). In the spring of 1922, therefore, larvae could be found in great abundance in cornstalks in the heavily infested portion of the New England area, and in considerably less abundance in the more sparsely infested areas, depending on the intensity of infestation. In other preferred host plants, such as cocklebur and barnyard grass, the larvae could be found in considerable numbers in a few localities, but in other host plants the number was too small to give satisfactory counts.

The counts of development were made to bear as close a relation as possible to the intensity of infestation in the various localities and in the several host plants. Most of the counts in cornstalks were taken in the heavily infested areas, a relatively smaller number being made in the more sparsely infested districts. A considerably smaller number of counts were taken in other host plants, and most of these were made in the most heavily infested weed areas, only a few being made in localities where these host plants or the larvae inhabiting them were less abundant.

All the counts for each day were then combined in a single weighted average for that day, and these daily averages, which may be termed "economic data," were smoothed by means of a moving average into a corrected curve of development.

PUPAE OF OVERWINTERING LARVAE:—The curve of occurrence of pupae was constructed in the manner described under "Overwintering larvae." The percentage of pupae present in the field on any

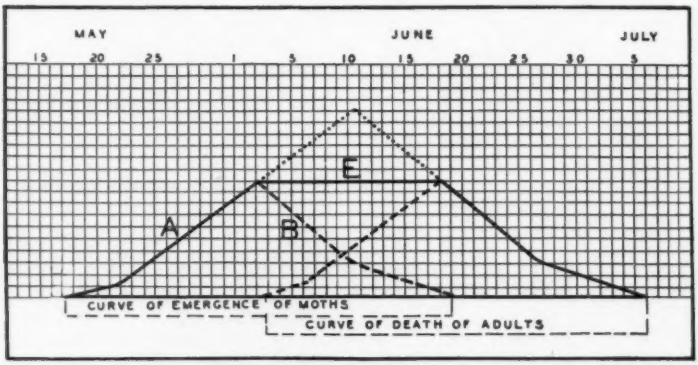

FIG. 2.—Detail of construction of the occurrence chart of "Adults of overwintering larvae" (fig. 1). The area bounded by the solid line is the area shown in Figure 1. A-B is the curve of emergence shown in the previous figure as a black area. The parallel curve to the right is the curve of average adult death, at all points following the emergence curve at a distance determined by the average length of life of adults. The dotted lines show, at their intersection, a date on which a possible maximum number of adults occurred in the field

given date would be the percentage of pupation, less the percentage of larvae and percentage of individuals that had emerged. This curve would run from zero to zero, a definite peak of occurrence of maximum pupae being possible because such a definite maximum was shown in the field counts.

ADULTS OF OVERWINTERING LARVAE.—A curve of emergence was constructed in the same manner as that described under "Overwintering larvae."

This curve was from 0 to 100 per cent of emergence, but in order to show occurrence, the curve increases (A) until 50 per cent of emergence occurred, and from that peak it decreases (B) until complete emergence was found. These curves, however, show only emergence, or the appearance of adults in the field, and not the occurrence of adults in the field, unless the length of life of the adult insects was taken into consideration. The average length of life for adults of this brood was found to be 15.9 days. Therefore it might

be expected that individuals emerging each day would survive in the field for an average of 15.9 days.

In Figure 2 the detail of the construction of the white portion of this chart is shown, the process being simply that of adding 15.9 days to each step in the advancement of curves A and B. This would present a chart with a flat top such as is shown in Figure 1. An extension of curves A and B, as shown in Figure 2, would show at their intersection a possible date on which a peak of occurrence of adults might have been found in the field. The writer prefers, however, to show peaks of occurrence only where such peaks were proved to have fallen on an exact date by field records, as in the case of pupae, and in the computed portions of the occurrence chart (fig. 2) to show maximum occurrence by a straight line as here done. Such a method of charting has the advantage of stressing the point that adults occur abundantly in the field over a certain period, rather than on a single day.

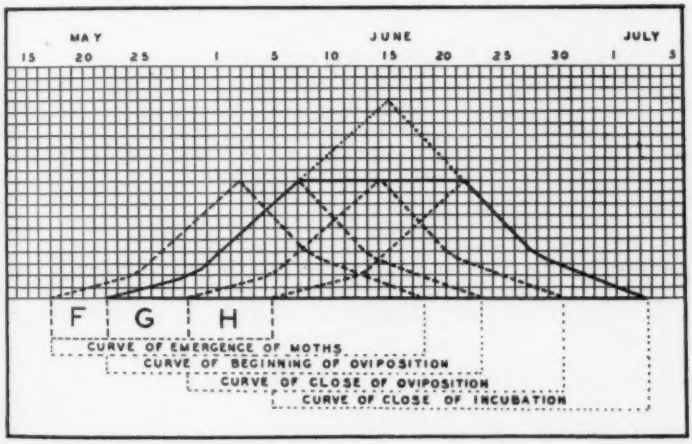

FIG. 3.—Detail of construction of the occurrence chart of "Eggs, first generation" (shown in fig. 1). "F" is the average preoviposition period, "G" the average oviposition period, and "H" the average incubation period. The area bounded by solid lines is the area shown in Figure 1. The dotted lines at their intersection show a date on which a possible maximum number of eggs occurred in the field

Since the insects in this stage of the life cycle can not be actually counted on a percentage basis in relation to other stages, the exact day of the occurrence of the maximum number of adults in the field is impossible to determine. Adults may live for a longer or a shorter period under field conditions than in the insectary, where it is impossible to reproduce all the conditions of freedom that would obtain in the field. The chart may be somewhat inaccurate, therefore, even if prepared with the greatest care, and in such a case the erection of a theoretical peak might magnify the error, whereas a chart of occur rence, such as Figure 1, constructed with a flat top showing a period of maximum occurrence, would at once admit of uncertainty as to the exact date of maximum occurrence, and serve as a warning th the adults appeared abundantly over a more or less protracted period and that control measures should be planned to meet this condition.

FIRST-GENERATION EGGS.—Eggs would appear in the field the preoviposition period "F" (4.9 days) later than adults, the curve of increase being comparable to the curve of increase of emergence (A). They would continue to appear in the field during the oviposition period of adults "G" (6.8 days), plus the incubation period "H" (7.4 days), the curve of decrease being comparable to the decrease in occurrence of adults (B). Figure 3 shows the details of the construction of the chart of occurrence of first-generation eggs. The curves of emergence (fig. 1, A and B) were first laid off (fig. 3, broken lines). Insectary studies showed that the preoviposition period of this brood of moths was 4.9 days. Therefore, eggs would begin to appear in the field on an average of 4.9 days later than the emergence of the first adults, and as adults continued to emerge, eggs would appear in a curve running parallel to the curve of emergence. A curve parallel to the curve of emergence (fig. 1, A and B) was therefore laid off 4.9 days later than the emergence curve, this being called the "curve of beginning of oviposition." Adults would continue to deposit eggs during the oviposition period, which was found to be on an average 6.8 days in insectary studies.

In the manner previously described, a third curve similar to the curve A–B in Figure 1 was laid off 6.8 days later than the curve of beginning of oviposition, this being called the "curve of close of oviposition." In exactly the same manner, a fourth curve was laid off the average incubation period (7.4 days) later than the curve of close of oviposition, because the eggs would continue to be present in the field until they hatched. This, the "curve of close of incubation," would indicate the hatching of the last eggs of any given series of moths, the falling portion showing the final disappearance of eggs in the field. The curve of increase of first-generation eggs in Figure 1 is, therefore, the rising curve called the "curve of beginning of oviposition" in Figure 3, whereas the curve of decrease in the chart of first-generation eggs in Figure 1 is the falling part of the "curve of close of oviposition" in Figure 3, these being the curves of appearance and disappearance of eggs in the field. The area contained within these curves is the period over which eggs were present in the field.

A possible date of the peak of occurrence of eggs in the field might be obtained by extensions of the increasing and decreasing curves of egg occurrence (fig. 3), the peak occurring on the date indicated by the intersection of these two extensions. The chart of first-generation eggs (fig. 1) is shown, however, with a flat top, for the same reasons as were set forth under the explanation of the chart of adults of overwintering larvae. If the adults occur over a period of 15.9 days in about the same abundance, the eggs would likewise occur over a certain period in nearly equal numbers. The straight line "G–H" in the chart of first-generation eggs in Figure 1 shows a period of greatest abundance of eggs in the field.

FIRST-GENERATION LARVAE.—In Figure 1 the occurrence of larvae in the field is computed (white area) until actual field counts of pupation begin (black area).

Larvae would begin to appear in the field the incubation period (represented by K) later than the first appearance of eggs, the curve of increase of larvae being uniform with the curve of appearance of eggs, but always the incubation period (7.4 days) later. If all the

larvae lived, the maximum number would not appear in the field until the last egg had hatched. But no such condition exists; on the contrary, a larger number perish, perhaps, particularly in the younger instars, than survive to transform. Therefore the facts of a constant mortality of larvae and a constant hatching of eggs balance one against the other for a period before eggs disappear from the field, and a point might be reached when a larger number of individuals perished than hatched daily. This would be particularly likely to happen when the occurrence of eggs is on the decrease.

Although a possible peak might be erected in the manner described under "Adults of overwintering larvae," the writer prefers to show a period of maximum occurrence beginning the incubation period later than the first date shown for maximum occurrence of eggs and extending in a straight line to the date of appearance of the first pupa, as shown in Figure 1.

The curve of decrease (shown in black) would begin with the record of first pupation. This curve is constructed in the manner described under "Overwintering larvae." Since a certain percentage of larvae (an average of about 14 per cent) failed to pupate and remained to pass the winter, the curve of decrease of larvae only reaches a point 14 per cent above zero and continues thereafter through the winter. A few larvae pupated through the fall, but the number was not sufficient to be indicated on the chart.

FIRST-GENERATION PUPAE (fig. 1).—This chart from records of field counts was constructed in the manner described under "pupae of overwintering larvae."

FIRST-GENERATION ADULTS (fig. 1).—This chart also was constructed in the same manner as that for "Adults of overwintering larvae." It is a combination of actual field records of emergence (black area) with the addition of length of life of adults (white area), as depicted in Figure 2.

SECOND-GENERATION EGGS.—This chart was constructed in the same manner as explained under "First-generation eggs," and illustrated in Figure 3.

SECOND-GENERATION LARVAE.—This chart also was constructed as explained under "First-generation larvae." Once the maximum had been attained it would continue through the winter as a straight line. Many larvae perished in the fall, winter, and spring, owing to natural agencies and to control measures such as the burning or plowing under of cornstalks. However, the chart (fig. 1) is of seasonal occurrence, and has nothing to do with population, so that as long as all the insects in the field were in the larval stage, the maximum would be continued. Thus, if in the spring there are in the field only a tenth as many larvae as were present the previous fall, the occurrence would nevertheless continue to be at the maximum until pupation is underway, for in each case all individuals of the species are in the larval stage, whatever their relative number may be.

SUMMARY

This paper sets forth the results of a study of the development of the European corn borer in the field in the New England area. Comparisons of this development in different food plants and in different localities were made in the two years, 1922 and 1923.

Considerable variation in the progress of development was found in these comparisons. On the whole, development was considerably more advanced on a certain date in 1922 than on the corresponding date in 1923, usually somewhat more advanced in weeds than in corn on the same date, and considerably more advanced in corn planted early than in corn planted somewhat later.

The records used in this study were of pupation and emergence only, and were obtained by a system of field counts, usually of 100 individuals each. Having available accurate information of the development of these stages of the European corn borer in New England, a chart was constructed showing the seasonal occurrence of the insect in 1922. It was found that by combining the accurate information of development obtained by the field counts with details of the life of adults as obtained by studies of caged individuals in the insectary, a chart of seasonal occurrence could be constructed. It is thought that such a chart is a fairly accurate guide to follow in planning measures and mapping out campaigns for the control of the insect.

TOXICITY OF ORGANIC COMPOUNDS TO THE SPORES OF PHYTOPHTHORA COLOCASIAE RAC.[1]

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INTRODUCTION

In recent years a large amount of work has been done on spore germination in relation to chemical and physical stimuli, but there has been comparatively little study of the relationship between the chemical constitution of a compound and its physiological action. It is not known, for instance, whether the toxicity of furfural is due to the aldehyde group which it contains, or to the presence of the unsaturated oxygen atom in the furan nucleus; whether there is always a similar physiologic response of the spores to the action of compounds, closely related chemically, such as aromatic and aliphatic aldehydes and alcohols; or whether the physiological response is regularly related to the physicochemical behavior, a conception held by those who believe there is a fundamental unity in the physiological responses of the living and the nonliving systems. These and a multitude of other matters suggest themselves, but the problem is so complex and its scope so large that it has here been possible to study only certain phases.

This investigation was undertaken to throw light on the group or radical in aldehydes and alcohols to which the toxicity of these compounds may be attributed. Simultaneously, a detailed and careful study of the toxicity of organic acids was made in order that knowledge on the comparative toxicity of these three classes of organic compounds might be gained, for any work that throws light on these problems will materially advance our knowledge of the physiological action of fungicides.

METHODS AND MATERIALS

ORGANISM

After experimentation with 20 species of the genus Phytophthora, cultures of which were obtained from various parts of this country and abroad,[3] *Phytophthora colocasiae* Rac. was selected as the species most suitable for this work, as it makes a fairly rapid growth on oatmeal agar and produces sporangia in abundance.

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[2] The writer is grateful to I. E. Melhus, Professor of Plant Pathology, Iowa State College, under whom this work was done, for many suggestions and criticisms. He is also indebted to R. M. Hixon, of the Department of Chemistry, Iowa State College, for critical reading of the manuscript.

[3] The writer expresses his thanks to William MacRae, Imperial Mycologist to the Government of India, to Leon H. Leonian of the West Virginia Agricultural Experiment Station, and to J. B. Kendrick of the Purdue University Agricultural Experiment Station, for supplying cultures of Phytophthora.

Phytophthora colocasiae Rac. is the fungus which parasitizes the leaves of *Colocasia antiquorum* Schott., and also causes a dry rot of the corm during the storage. The fungus produces both sexual and asexual spores. The asexual spores are very large. When formed on the leaf they measure from 38 to 60 by 18 to 26 μ in diameter. In culture, however, the range of variation is much wider. The sporangia germinate readily in distilled water, either by putting out a germ tube or by the formation of zoospores, which in turn germinate by a hypha. These two modes of germination are entirely controlled by the effect of temperature on the spores. Temperatures ranging from 10° to 25° C. favor the formation of zoospores in the sporangia, which, if the culture is not older than 7 days, germinate as high as 95 to 100 per cent. At high temperatures, from about 25° to 32° C., germination of the sporangia occurs exclusively by a hypha, which may form a mycelium or give secondary and sometimes tertiary sporangia after a short growth. Under the most favorable conditions of higher temperatures, from 29° to 32°, the sporangia will produce germ tubes in water cultures in three hours or less.

Two stock cultures of the fungus were made every day, and these were allowed to grow at room temperature for a week. Occasionally, when the temperature of the room fell below 22° C., the growth of the fungus was slowed down and the stock cultures were not ready for use until after eight, occasionally nine. days. At this time the sporangia were fully mature and were produced in great abundance. To obtain uniformity, it has been the custom to use spore material from a single tube in making an entire series of cultures. Direct inoculation of the culture drop was effected by removing a little mycelium with a thin flat-pointed platinum needle and shaking it gently in the drop. The spores fall off very readily, if the culture is not older than 7 days.

STOCK SOLUTIONS OF CHEMICALS

The organic compounds used were of the highest purity obtainable.

Stock solutions of the chemicals were prepared in molar (normal in the case of acids) concentrations, the strength of the stock solutions depending upon the degree of solubility of the compounds in water. In preparing stock solutions chemicals were weighed in a clean pyrex glass beaker on a chainomatic balance, which corrected the weight of the chemicals to the fourth decimal place. Most of the organic compounds do not go into solution in cold water readily, and they were dissolved in hot water, care being taken that the temperature of the hot water always remained far below the boiling point of the compound. The volumetric flasks containing the dissolved chemicals were immersed in water at 20° C., and enough water was added to the flasks to make the required volume. The water used in making the stock solutions was doubly distilled over a hard glass condenser from a solution of potassium permanganate in a hard glass flask.

In the case of alcohols, percentage solutions were made, and hence molar stock solutions were not prepared. As some of the alcohols were expensive, a method was worked out whereby fresh experimental solutions of any alcohol could be prepared by pipetting out 0.1 c. c. of a certain alcohol and then adding distilled water.

In this way not more than 3 or 4 c. c. of each alcohol were required for a sufficiently large number of experiments to determine fairly exactly the limits of toxicity.

METHOD OF CULTURE

The various dilutions of the compounds to be tested were made in pyrex glass beakers of 50 c. c. capacity. Each beaker was provided with a glass rod having a round, blunt point. Special care was taken to make the blunt points on the rods as nearly alike as possible, for the reason that variable shapes may give culture drops of variable size, thus giving erratic results.

For making cultures, Van Tieghem cells were used, which were cemented to ordinary slides by means of refined beeswax. All cultures were made in duplicate. A drop of the solution to be tested was placed on a clean cover glass, which was then sealed on to the top of the cell with petrolatum. One to two drops of the same solution were placed in the bottom of the cell. The slides were then ready to be removed to an incubator.

For making cultures of alcohols, watch glasses of a type devised by the Bureau of Plant Industry, United States Department of Agriculture, were used, since in hanging-drop cultures the low surface tension of the alcoholic solutions used tended to spread the drop on the cover glass. In cross section these watch glasses are similar to the Syracuse watch glasses, but much smaller in size, viz, 27 mm. diameter by 8 mm. high, and have, unlike the Syracuse glasses, ground beveled edges. In making experimental cultures, 6 drops of the alcoholic solution to be tested were placed in the center of the watch glass and charged with spores.

In attempting to determine exactly the concentration of a compound which would inhibit germination of the spores, the procedure was as follows: 1 c. c. of the stock solution was pipetted out in a beaker, to which 9 c. c. of distilled water was added. The solution thus obtained was in turn diluted to one-tenth of its strength, and the process was repeated until a solution which contained 1 part of the stock solution to 100,000 parts of water was obtained. All these dilutions were charged with spores, and after an incubation period of 18 hours the cultures were examined for the lowest concentration of the compound that inhibited spore germination. Let this be 0.001 M. Then a set of 10 cultures, ranging from 0.001 M to 0.0001 M, was made in duplicate. This made it possible to determine the concentration at which spore germination was inhibited. Suppose the concentration of inhibition was found to be 0.0006 M. Another set of five cultures, ranging from 0.0004 M to 0.0008 M, was prepared. This was repeated twice, and if the concentration of inhibition remained the same, the findings were taken as correct.

CLEANING GLASSWARE

After the cultures were examined, the Van Tieghem cells, cover glasses, and microscopic slides were boiled in two changes of water to remove beeswax and petrolatum, and then soaked in xylol for 10 to 15 minutes. They were then washed in much the same manner as that described by Clark (*3*),[4] except that distilled water was always used in washing cover glasses. All pipettes were washed by

[4] Reference is made by number (italics) to "Literature cited," p. 1095.

forcing tap water through them for two hours and then washing them in distilled water.

The watch glasses were soaked in xylol for 15 minutes to remove petrolatum, then washed freely under the tap for the same length of time, and finally rinsed in 95 per cent alcohol. They were then washed in several changes of distilled water, wiped, and sterilized in an oven for 30 minutes.

EXAMINATION OF CULTURES

In determining the effect of various organic compounds upon the sporangia, the best index was the occurrence or nonoccurrence of germination after periods of 6 and 18 hours. In the case of indirect germination, any renewed activity in protoplasm, besides the formation of true zoospores, was regarded as germination. The renewed activity of protoplasm, under the influence of deleterious agents, manifests itself in the streaming out of the intersporangial protoplasm, which finally breaks up into small granular masses. Direct germination occurs by the production of a germ tube, although in some cases the mycelium from a germ tube may give secondary and tertiary sporangia. Under the debasing influence of toxic agents, germ tubes become very slender and appear stunted in growth.

The concentrations of toxic agents in which the sporangia failed to germinate were divided into two classes: (1) The *critical concentration*, or that lowest concentration of a deleterious agent which made germination doubtful or which had an inhibiting influence without the least visible signs of plasmolysis or other abnormalities in the sporangia; (2) the *lethal concentration*, or that lowest concentration of a toxic agent which brought about slight or pronounced plasmolysis of a few or many sporangia, a fact that was taken as a sure index of the death of the spores. In considering results on the lethal concentrations of various organic compounds, a difficulty was encountered in that resistance of the spores showed great variation; for instance, a concentration of a toxic agent will at one time produce the plasmolyzed condition of the sporangia, while at another time there will be no visible sign of any abnormality. Caution should therefore be exercised in accepting these results, for the boundary line between the critical and lethal concentrations is so narrow that a slight alteration in the resistance of the spores may throw the results from one concentration to the other.

The cultures for direct germination were kept in an electric incubator, in which the temperature was maintained at 30° to 31° C. For indirect germination, the cultures were kept in an automatically controlled refrigerator, cooled by sulphur dioxide. This refrigerator had various compartments, which registered temperatures varying from 4° to 16°; in fact, still lower temperatures could be obtained by regulating the cooling machine. The cultures were kept in a compartment registering 11° to 12°.

COMPARATIVE TOXICITY OF ALCOHOLS

Before proceding with the discussion of the experimental data, a brief resume of the important literature on the toxicity of alcohols will show the present state of knowledge of this subject.

Stadler (*18*), using *Staphylococcus aureus*, *Bacterium coli*, and *Bacillus pyocyaneus*, and Morgan and Cooper (*13*), working with

Staphylococcus pyogenes aureus and *Bacillus typhosus*, have shown that the toxicity of the normal alkyl alcohols increases as the homologous series is ascended, with the exception that methyl and ethyl alcohols, according to the latter writers, showed the same toxicity. However, Vandevelde (*19*) and Förster (*4*) contend that the failure of methyl alcohol to behave in accordance with the rule of increasing molecular weight is largely due to impurities. The conclusions of Stadler (*18*) concerning bacteria are further supported by the work of Vernon (*20*) with the tortoise, Kuno (*8*) with the rabbit, and Macht (*11*) with the rabbit and the frog. Kamm (*7*), testing the toxicity of various normal primary alcohols upon paramecia, has found that, with the exception of methyl alcohol, if the toxicity of ethyl alcohol be taken as unity, the successive members may be arranged as to toxicity according to the following geometrical progression: 3, $3,^2$ $3,^3$ $3,^4$ etc.; in other words, the molar toxicity of any member in a homologous series is three times that of the succeeding member.

Vernon (*20*) and Macht (*11*), in the case of normal propyl, normal butyl, and normal amyl alcohols, and Morgan and Cooper (*13*) in the case of normal propyl and normal butyl alcohols, showed that the normal alcohols were more toxic than their corresponding isomers. Moreover, Vernon (*20*) found that secondary and tertiary butyl alcohols were toxic in this order of mention, but both were less toxic than isobutyl.

Carlier (*1*, *2*) studied the effects of allyl isothiocyante, allyl sulphide, allyl acetate, and allyl alcohol, upon respiration and blood pressure in the rabbit and the frog, and concluded that the allyl compounds owe their toxicity to the allyl radical.

Ruediger (*17*, *p. 475–477*), working with the anthrax organism, found that glycerol exerts more feeble germicidal action at 15° C. than at 30° to 35°. Glycerol also exerts a specific toxic influence on cholera, diptheria, and plague organisms. Winslow and Holland (*23*), using varying concentrations of glycerol on *Bacterium coli*, concluded that a 9 per cent solution of the alcohol did not exercise any appreciable influence on the viability of *Bacterium coli*, but solutions between 28 and 100 per cent strength showed progressively increasing toxic action on the organism.

Practically no work has been done on the toxicity of alcohols in relation to plant pathogenes, especially the parasitic fungi.

TOXICITY TRIALS

Typical alcohols from the aliphatic and the aromatic series were investigated. In the present case only indirect germination of the sporangia of *Phytophthora colocasiae* was employed as a criterion of the relative toxicity of the alcohols.

The toxic action of the 13 alcohols of the aliphatic series has been summarized in Table I. The first group is that of the monohydric alcohols. It will be seen from the table that a 7.9 per cent solution of methyl alcohol prevents the liberation of zoospores, while ethyl alcohol of 6.3 per cent strength exerts the same effect. These results were a little surprising, since it was expected that methyl alcohol, like first members of the homologous series, would stand out by itself in its physiological action, and would prove more toxic to the spores than is indicated in the table.

TABLE I.—*Toxic effect of alcohols on the spores of Phytophthora colocasiae Rac*

Compound	Number of experiments	Number of cultures	Indirect germination	
			Critical concentration, per cent	Critical concentration, molar [a]
A Aliphatic alcohols:				
a. Monohydric alcohols—				
I. Saturated alcohols—				
Methyl alcohol	5	32	7.9	1.96 (15° C.)
Ethyl alcohol	5	11	6.3	1.1
Normal propyl alcohol	5	28	3.1	.41
Isopropyl alcohol	5	28	6.5	.853
Normal butyl alcohol	3	18	1.5	.163
Isobutyl alcohol	7	36	1.8	.195 (15° C.)
Secondary butyl alcohol	6	30	3.2	.357
Tertiary butyl alcohol	5	24	6.3	.668
Normal amyl alcohol	3	16	[b] 1.2	[b] .111
Isoamyl alcohol	4	14	1.0	.091
II. Unsaturated alcohol—				
Allyl alcohol	7	34	.0005	.000073
b. Dihydric alcohol—				
Ethylene glycol	7	28	6.5	1.16
c. Trihydric alcohol—				
Glycerol	3	14	6.2	.872
B. Aromatic alcohols:				
I. Saturated alcohol—				
Benzyl alcohol	3	14	.2	.0192
II. Cyclo-olefine—				
Furfuralcohol	9	46	.2	.0231

[a] To correspond with critical concentrations in per cent, molarity of these solutions is calculated approximately on the basis of the specific gravity of different alcohols at 20° C., unless otherwise stated.
[b] Amyl alcohol not in solution.

Normal propyl alcohol is about twice as toxic as ethyl alcohol, inhibiting the formation of zoospores in 3.1 per cent solution. On the other hand, isopropyl alcohol, which prevents indirect germination of the spores in 6.5 per cent solution, is a little less toxic than ethyl alcohol. In this connection it is interesting to note how the physiological action of a compound is modified on account of a change in its structural constitution. A satisfactory explanation of such facts would, no doubt, materially advance our knowledge of physiological chemistry.

The behavior of normal butyl alcohol and its isomers is still more interesting. A 1.5 per cent solution of normal butyl alcohol will prevent the formation of zoospores; that is, normal butyl alcohol has 4.2 times the toxicity of ethyl alcohol. Isobutyl alcohol, though still rather toxic, has its poisonous effect reduced to about three-fourths that of normal butyl, or its toxic action is only 3.5 times that of ethyl alcohol. Secondary butyl alcohol shows a still greater fall in toxic value. It is approximately one-half as toxic as normal butyl alcohol, the strength of the alcohol inhibiting indirect germination of the spores being 3.2 per cent. The toxicity of tertiary butyl alcohol is so much reduced that it exactly corresponds with that of ethyl alcohol. Thus, isobutyl alcohol is less toxic than normal butyl, secondary butyl less than isobutyl, and tertiary butyl less than secondary butyl.

Normal amyl alcohol and its isomer were next investigated. A 1.2 per cent solution of normal amyl alcohol prevents the liberation of zoospores, while a 1 per cent solution of isoamyl exerts the same effect. The writer does not place much reliance in these results, as it was not possible to obtain even a 1 per cent solution of normal

amyl alcohol, although it is reported soluble to the extent of 2.72 gm. in 100 c. c. of water at 22° C. Even after a gentle warming, normal amyl alcohol did not go into solution, but floated on the surface of water as tiny droplets, which could be seen easily by holding the beaker against the light.

Allyl alcohol, an unsaturated monohydric alcohol, is the most toxic of the alcohols investigated. A 0.0005 per cent solution of the alcohol prevents the formation of zoospores. Compared with ethyl alcohol, it is 12,600 times more toxic. The presence of a double bond leads to an increased activity of the alcohol, and it seems that this chemical activity accelerates the toxic action of the compound. Moreover, it has been shown by Carlier (*2*) that the toxicity of the alcohol is due to the allyl radical that it contains.

Ethylene glycol, or glycol, is the simplest glycol known, and corresponds with ethyl alcohol. It is of about the same toxicity as ethyl alcohol, the critical concentration for indirect germination of the spores being 6.5 per cent. Glycerol, the simplest trihydric alcohol, is also not very toxic, its critical concentration being 6.2 per cent.

Only two alcohols from the aromatic series were investigated. Benzyl alcohol prevents the liberation of zoospores in 0.2 per cent solution. It has about 31 times the toxicity of ethyl alcohol. The toxicity of benzyl alcohol is mostly due to the benzene nucleus. Furfuralcohol, like benzyl alcohol, inhibits indirect germination of the spores in 0.2 per cent solution. Its toxicity may be due to the unsaturated oxygen atom in the furyl radical.

COMPARATIVE TOXICITY OF ALDEHYDES

Formaldehyde is one of the most important fungicides, but very little is known regarding its action. So it seemed desirable to investigate a number of aldehydes, both aliphatic and aromatic, in order to throw light on the group or radical in the aldehyde molecule to which the toxicity of aldehydes in general may be attributed. A review of the literature shows that comparatively little study has been made of this problem, especially in its relation to the fungi.

Stadler (*18*), working with *Staphylococcus aureus*, *Bacterium coli*, and *Bacillus pyocyaneus*, showed that in aliphatic aldehydes the lower homologues decreased in toxicity with increasing molecular weight. However, Vernon (*20*), using depression of the heart beats of the tortoise as a criterion of toxicity, found that the toxicity of the four aldehydes investigated had no relationship to molecular weight. He has given the relative toxicity of the aldehydes as follows: Propionaldehyde, 1.0; acetaldehyde, 1.2; isobutyraldehyde, 1.8; and formaldehyde, 40.

Warburg (*22*) tested the action of aldehydes in modifying the oxidative processes of the young goose erythrocytes, and found that the following molar concentrations caused a 30 to 70 per cent reduction in oxygen consumption: Formaldehyde, 0.001; acetaldehyde 0.013; propionaldehyde, 0.01; normal butyraldehyde, 0.008; isobutyraldehyde, 0.01; isovaleraldehyde, 0.0035; furfural, 0.003.

Loeb (*9*) observed that the subcutaneous injection of rabbits with the aliphatic aldehydes, formaldehyde, acetaldehyde, normal valeraldehyde, enanthaldehyde, and citral, resulted in arterial necrosis, which was not produced by furfural and aromatic aldehydes.

Verzár and Felter (*21*) found that formaldehyde, glyoxal, and acrolein always produced the typical veratrine contraction of the transversely striated muscles, while acetaldehyde gave the same reaction three out of five times, paraldehyde four out of six, and glyceryl aldehyde two out of five. All other aldehydes failed to give the reaction.

Moore (*12*) tested a number of organic compounds for toxicity to the house fly, and found that of all the aldehydes used, salicylic aldehyde was the most toxic. Its toxicity was 19 times that of furfural and 3.5 times that of benzaldehyde.

McGuigan (*10*) investigated the action of furfural on bacteria, yeasts, and goldfish. He found that the phenol coefficient of furfural, measured from its bactericidal action, was 0.26. A 2 per cent solution of furfural entirely inhibited the action of yeasts on dextrose. The toxicity of furfural to goldfish was one-half that of phenol and about one-third that of formaldehyde.

TOXICITY TRIALS

Eleven aldehydes from the aliphatic and the aromatic series have been investigated. Direct and indirect germination of the sporangia of *Phytophthora colocasiae* was used as a criterion of the relative toxicity of aldehydes.

It will be seen in Table II that formaldehyde[5] exerts a greater inhibitory effect on the formation of zoospores than on the production of germ tubes. It inhibits the liberation of zoospores in 0.0009 M solution, while the critical concentration for direct germination is 0.002 M.

TABLE II.—*Toxic effect of aldehydes on the spores of Phytophthora colocasiae Rac.*

Compound	Formula	Number of experiments	Number of cultures	Indirect germination		Direct germination	
				Critical concentration, molar	Lethal concentration, molar	Critical concentration, molar	Lethal concentration, molar
Aliphatic aldehydes:							
Formaldehyde	$H.CHO$	8	80	0.0009	.....	0.002	0.004
Acetaldehyde	$CH_3.CHO$	5	74	.05	0.06	.06	.07
Propionaldehyde	$C_2H_5.CHO$	3	60	.07	.....	.07	.1
Normal butyraldehyde	$C_3H_7.CHO$	3	56	.05	.....	.08	.1
Glyoxal	$CHO.CHO$	4	50	.005	.007	.006	.007
Aromatic aldehydes:							
Benzaldehyde	$C_6H_5.CHO$	2	16	[a].02	.....	[a].02	.....
m-Nitrobenzaldehyde	$NO_2.C_6H_4.CHO$	4	38	.005	.....	.008	.009
o-Nitrobenzaldehyde	$NO_2.C_6H_4.CHO$	4	40	.005	.....	.008	.009
p-Hydroxybenzaldehyde	$OH.C_6H_4.CHO$	4	46	.003	.004	.005	.006
Protocatechuic aldehyde	$(OH)_2.C_6H_3.CHO$	3	24	.003	.004	.003	.005
Furfuraldehyde	$C_4H_3O.CHO$	4	80	.04	.....	.05	.01

[a] Sporangia germinated.

The critical concentrations of acetaldehyde for direct and indirect germination of the spores are 0.06 M and 0.05 M, respectively. In other words, for indirect germination the toxicity of formaldehyde is

[5] The formaldehyde used in these experiments was analyzed in the Physical Chemistry Laboratories at Ames, Iowa, by F. Schulze, and was found to contain 37.7 per cent of formaldehyde by volume and 34.68 per cent by weight.

about 55.5 times that of acetaldehyde, while for direct germination its action is only 30 times stronger. The toxicity gradient for acetaldehyde showed an abrupt decline, for while a 0.06 M solution of the aldehyde permits the production of germ tubes, the sporangia are badly plasmolyzed in 0.07 M concentration. A similar condition occurs in the case of indirect germination.

Propionaldehyde acts very much like acetaldehyde, although it is a little weaker than the latter. Its critical concentration for direct and indirect germination is 0.07 M. There is a gradual rise in the toxicity of the aldehyde, and the sporangia do not exhibit any plasmolysis until a 0.1 M concentration of the aldehyde is reached.

Normal butyraldehyde, like formaldehyde, shows a greater inhibitory action to the formation of zoospores than to the production of germ tybes. Its inhibitory concentration for indirect germination is 0.05 M, while the production of germ tubes is retarded at 0.08 M. The toxicity of the aldehyde increases gradually with concentration, so that there is a gradation from true germination through inhibition to plasmolysis of the sporangia. Stock solutions deteriorate on long standing at room temperature, and it is therefore advisable not to prepare stock solutions long before they are to be tested.

Glyoxal or glyoxaldehyde, contains two aldehyde groups without having any alkyl radical attached to them. It is about 10 times more toxic than any other aliphatic aldehyde, except formaldehyde. Its critical concentrations for direct and indirect germination of the spores are very nearly the same, being 0.006 M and 0.005 M, respectively. Glyoxal is about one-half as toxic as formaldehyde. In the case of direct germination, the toxicity curve shows a rather sharp decline, as the concentrations for inhibition of germination and death of the spores coincide. Hence, correctly speaking, there is no critical concentration for direct germination in this case.

Of the aromatic series, six aldehydes were investigated for toxicity, the results being summarized in Table II. Benzaldehyde is reported soluble to the extent of 0.33 gm. in 100 c. c. of water. In other words it is possible to prepare only a 0.025 M solution. However, the stock solution prepared was of 0.02 M strength, a concentration which allowed both direct and indirect germination.

Meta- and ortho-nitrobenzaldehydes were next investigated. These two aldehydes are very nearly as toxic as glyoxal, and are 10 times more toxic than any aliphatic aldehyde, excepting formaldehyde. These aldehydes exert a greater inhibitory action on indirect than on direct germination. Paranitrobenzaldehyde could not be investigated, as it is only slightly soluble in hot water. Parahydroxybenzaldehyde is about twice as toxic as meta- or ortho-nitrobenzaldehyde. Its critical concentrations for direct and indirect germination are 0.005 M and 0.003 M, respectively. Protocatechuic aldehyde, which has its critical concentration at 0.003 M, shows about the same toxicity as parahydroxybenzaldehyde. Evidently, then, the presence of two hydrozyl groups in protocatechuic aldehyde does not enhance the toxicity of the compound to any great extent. It should also be noted that in all of the four substituted benzaldehydes investigated the toxicity curve shows a rather sharp fall similar to that found in glyoxal.

Furfural is not very toxic to the spores, the concentrations inhibiting direct and indirect germination being 0.05 M and 0.04 M, respectively. Furfural shows the same toxicity to the spores as do the aliphatic aldehydes, except formaldehyde. It also shows a gradual killing effect, increasing steadily with concentration.

COMPARATIVE TOXICITY OF ORGANIC ACIDS

The toxic effect of organic acids on fungous spores has not been studied extensively. In fact, there is very little information on this subject in the literature, except the work of Kahlenberg and True (*6*), who investigated the toxicity of a number of inorganic and organic compounds to the seedlings of *Lupinus albus* L.

TOXICITY TRIALS

Summary of Table III

From Table III it appears that formic acid is the most toxic acid of the fatty acid series to the sporangia of *Phytophthora colocasiae*. Its critical concentration for direct germination of the spores is 0.0025 N, which is about one-third the critical concentration of acetic acid, the second in the order of toxicity. With regard to indirect germination of the spores, a similar relationship holds, although in this case the critical concentration of formic acid is one-seventh that of acetic acid and one-fifth that of isovaleric acid, which here happens to be the next in the order of toxicity.

Table III.—*Toxic effect of the monocarboxylic fatty acids and the substituted acids on the spores of Phytophthora colocasiae Rac.*

Compound	Number of experiments	Number of cultures	Indirect germination		a at 10° C.[a]	$H^+ + R^{-b}$	HR[c]
			Critical concentration, normal	Lethal concentration, normal			
Saturated carboxylic acids:							
Formic acid	4	50	0.001	0.002			
Acetic acid	6	64	.007		[d]5.4 (9.2)	0.000378	0.00662
Propionic acid	6	72	.008	.01	4.02(6.9)	.000321	.00767
Normal butyric acid	3	40	.008	.01	4.42(9.4)	.000353	.00764
Isobutyric acid	3	32	.009		4.00	.00036	.00864
Normal valeric acid	3	36	.008	.009			
Isovaleric acid	3	36	.005		5.5(15)	.000275	.00472
Unsaturated carboxylic acids:							
Crotonic acid	3	28	.003	.005	7.6(12)	.000228	.00277
Halogen-substituted acetic acids:							
Monochloracetic acid	4	46	.001	.002	67	.00067	.00033
Dichloracetic acid	5	78	.001	.002	99.9	.000999	.00001
Trichloracetic acid	4	58	.001	.002	99.9	.000999	.00001
Monobromacetic acid	4	60	.0004	.007			
Hydroxycarboxylic acids:							
Glycollic acid	3	44	.0007				
Lactic acid	6	64	.0007	.0008			

TABLE III.—*Tonic effect of the monocarboxylic fatty acids and the substituted acids on the spores of Phytophthora colocasiae Rac.*—Continued

Compound	Number of experiments	Number of cultures	Direct germination		α at 30° C.[a]	$H^+ + R^-$[b]	HR[c]
			Critical concentration, normal	Lethal concentration, normal			
Saturated carboxylic acids:							
Formic acid	4	50	0.0025	0.003	35.4 (25)	0.000885	0.00161
Acetic acid	6	64	.007	.01	5.4	.000378	.00662
Propionic acid	6	72	.008	.009	4.02	.000321	.00767
Normal butyric acid	3	40	.008	.01	4.24	.000339	.00766
Isobutyric acid	3	32	.009	.01	3.7	.000333	.00866
Normal valeric acid	3	36	.008	.009			
Isovaleric acid	3	36	.009		4.07	.000366	.00863
Unsaturated carboxylic acids:							
Crotonic acid	3	28	.003	.004	7.68	.00023	.00277
Halogen-substituted acetic acids:							
Monochloracetic acid	4	46	.002	.003	56.3	.00112	.00088
Dichloracetic acid	5	78	.002	.003	98.5	.00197	.00003
Trichloracetic acid	4	58	.002	.003	99.3	.00198	.00002
Monobromacetic acid	4	60	.0005	.0007			
Hydroxycarboxylic acids:							
Glycollic acid	3	44	.001	.002	31.8	.000318	.000682
Lactic acid	6	64	.001	.002	30.6	.00306	.000694

[a] The value of α is the percentage of an acid ionized at its critical concentration.
[b] $H^+ + R^-$ represents the concentration of the H-ions and the anions at the critical concentration.
[c] HR Represents the concentration of the undissociated molecules at the critical concentration.
[d] The number in parentheses represents the temperature on the centigrade scale, at which the value of α is determined.

It is evident from Ostwald's dilution law and formula that of two acids the one which is more highly ionized has a greater constant. Thus formic acid, which is the most highly ionized of the fatty acids, is stronger than any other in the series. Acetic acid, the second in the order of toxicity as well as the extent of ionization, is a little stronger than propionic acid. With regard to direct germination, there seems to be but little difference in the critical concentrations of all the normal fatty acids, except formic; but for indirect germination the critical concentration of acetic acid is a little higher than that of isovaleric acid, which in the case of direct germination appears to be one of the least toxic; otherwise the differences among other acids are not great enough to be of significance. This is, however, in accordance with what would have been expected, considering that these acids are ionized to approximately the same degree, and their alkyl radicals do not appear to differ markedly in chemical nature.

The relatively greater toxic effect of formic acid may also be due to its structural peculiarity. According to its structure, formic acid contains an aldehyde group; in other words, it is the only organic acid that has in the molecule an unsaturated hydrogen atom, which is very sensitive to oxygen. This, coupled with the fact that formic acid at its critical concentrations is more highly ionized than any other saturated monocarboxylic fatty acid, may account for its relatively greater toxicity.

According to Ostwald's (*15*) determination, 1 gram molecular weight of acetic acid in 1,024 liters of water is 12.66 per cent ionized; but in the case of direct and indirect germination the critical concentrations of this acid contain only 1 gram molecular weight of it in about 143 liters of water; that is, at these concentrations the acid is about 5.4 per cent ionized (by interpolation). It therefore follows that since

such a small portion of the acid is ionized, its toxic properties can not be due entirely to the influence of the H-ions or to that of the anions. Hence it is the undissociated molecules which partly seem to be responsible for the toxic effects of acetic acid.

For direct and indirect germination of the spores, the critical concentration of propionic acid is 0.008 N. According to Jones's (*5, p. 87–144*) determination, the acid at the critical concentration is about 4 per cent ionized. Hence the toxicity of the acid does not seem to be determined entirely by the poisonous action of the H-ions or the anions, but is due, in part, to the effect of the undissociated molecules.

The electrolytic dissociation of normal buytric and isobutyric acids is very nearly the same, which is characteristic of a large number of isomeric compounds. The poisonous action of these two acids corresponds with the degree of their electrolytic dissociation, normal butyric acid, which is slightly more ionized than its isomer, being more toxic than isobutyric. It is of interest to note in this connection that a change in the structural constitution of the two acids has not modified their physiological action, except for a slight difference in the electrolytic dissociation. However, it is to be seen later that this does not hold true in all cases of isomerism. The concentrations of normal butyric and isobutyric acids which do not permit direct and indirect germination of the spores are 0.009 N and 0.008 N, respectively. According to Jones's (*5, p. 87–144*) determination, normal butyric and isobutyric acids, at their critical concentrations, are about 4 per cent ionized. A study of their sodium salts will determine the extent to which the anions and the undissociated molecules influence the toxicity of the acids. However, the effect of the H-ions in contributing to the total toxicity is about the same as in acetic acid.

Unlike normal butyric acid, the ionization constant of normal valeric acid is less than that of isovaleric acid. It is to be expected, then, that normal valeric acid will be less poisonous to the spores than its isomer. This is true in the case of indirect germination, where the critical concentration of normal valeric acid is 0.008 N, and that of its isomer 0.005 N; but the order of the toxicity of the two acids is reversed in the case of direct germination. Here normal valeric acid inhibits germination at 0.008 N, while its isomer exerts the same effect at 0.009 N. Interpolating Jones's (*5, p. 87–144*) figures, the electrolytic dissociation of isovaleric acid at 15° C. and at the dilution 200 (the critical concentration for indirect germination) is about 5.5 per cent, and at 30° C. about 5.2 per cent, a significant difference considering the amount of the acid ionized. But it is very doubtful whether this difference in the degree of ionization accounts for the decrease in the strength of the acid at 30° C., which is a little less than one-half of its strength at 15°. The experiment has not been repeated a sufficient number of times to exclude all chances of error.

Of the unsaturated carboxylic fatty acids, crotonic acid was investigated. The critical concentration for direct and indirect germination of the spores is 0.003 N. According to Jones's (*5, p. 87–144*) determination, the acid at its critical concentrations is about 7.6 per cent ionized. It seems that the effect of the H-ions in determining the toxicity of the acid at its critical concentrations is not very great, since only about one-thirteenth of the total amount of the acid

present in the solution is ionized. The presence of a double bond in the acid seems to accelerate its toxicity.

The three chloro-acetic acids differ greatly from acetic acid as to toxicity, in that they are about seven times more toxic than the latter. This difference, however, is due to the fact that the introduction of chlorine or any other halogen into the positive methyl radical of acetic acid renders the radical negative, and brings about a marked change in its properties. This change manifests itself partly in an increase in the ionization constants of these acids. For instance, the replacement of one hydrogen atom in acetic acid by one chlorine atom increases the constant of the acid 86 times, while the constant of dichloracetic acid is about 33 times as great as that of monochloracetic acid, and that of trichloracetic acid 23.5 times as great as that of the disubstituted acid. Monobromacetic acid has about 80.6 times the constant of acetic acid. It seems reasonable, then, to assume that the order of toxicity would be tri-, di-, and monochloracetic acid, followed closely by monobromacetic acid. But such a condition is obtained only when the toxicity of these acids is solely dependent upon the concentration of the H-ions in the solution.

Since the introduction of a halogen into the methyl radical of acetic acid also results in the increased toxicity of the undissociated molecules, it seems that the toxic properties of the halogen-substituted acetic acids are due to the combined effects of the H-ions and the undissociated portion. Moreover, as ionization advances, the toxic influence is exerted by a greater concentration of the H-ions, more than by the presence of the undissociated molecules; conversely, the lower the ionization, the greater the toxic effect due to the undissociated portion. Clark (*3*), using potassium salts of the three chloro-acetic acids, worked out the toxic values of their undissociated molecules, showing that the replacement of one hydrogen atom in the methyl radical of acetic acid doubles the toxic properties of the un-ionized portion, while the replacement of two hydrogen atoms trebles its toxicity, and the replacement of all three hydrogen atoms increases its toxic value about five times.

That the three chloro-acetic acids behave alike as respects their toxic action now seems easy to explain. In the case of indirect germination, monochloracetic acid, which is about 67 per cent ionized at its critical concentration (0.001 N), has about 33 per cent more of the un-ionized portion than either of the other two chloroacetic acids, which are about 99.5 per cent ionized. In other words, the toxicity of monochloracetic acid is determined by the combined effects of the H-ions and the undissociated molecules; whereas in di- and tri-chloracetic acids the influence of the H-ions alone seems to account for their toxic properties, for at this dilution (0.001 N) the two acids are so completely ionized that the amount of the dissociated portion is rather too small. Thus the slight toxicity of monochloracetic acid due to low degree of electrolytic dissociation is so supplemented by the toxic properties of the undissociated portion that the resultant of these two factors gives a toxic value equal to that for the other two acids.

The concentrations of the chloro-acetic acids inhibiting the production of germ tubes is 0.002 N, which is twice the concentration which prevents the liberation of zoospores. Moreover, it should be noted that at the critical concentrations of the acids, both for direct

and indirect germination, most of the sporangia were badly plasmolyzed, and that it was only a very few sporangia that germinated.

For direct germination of the spores, monobromacetic acid is four times as toxic as the chloro-acetic acids, while for indirect germination it is only about two and one-half times as toxic. Data on the electrolytic dissociation of the acid for dilutions higher than 1,024 were not available, although it seems quite safe to assume that the dissociation of the acid at the dilutions 2,500 and 2,000 is not far from complete. Hence the toxic effects at these high dilutions are exerted more by the concentration of the H-ions and the anions in the solution than by that of the undissociated portion. However, it appears hardly likely that the H-ions will exert any great effect at these high dilutions. For an explanation of the toxicity of the acid the aid of the sodium salt of the acid must be invoked, in order to show the extent to which the anions influence the total toxicity.

Passing to the hydroxycarboxylic fatty acids, we come to glycollic acid, which is a hydroxyacetic acid. The introduction of a hydroxyl group into acetic acid increases the dissociation constant of the acid seven and seven-tenths times. The liberation of zoospores is inhibited at 0.0007 N, which is about one and one-half times less than the concentration which allows a few sporangia to germinate by germ tubes. For direct germination there does not seem to be a critical concentration of the acid, since no concentration was found to inhibit germination of the spores without showing signs of plasmolysis.

Lactic acid is a hydroxypropionic acid, and has about 9.7 times the dissociation constant of propionic acid, due to the presence of a hydroxyl group. The critical concentrations of this acid are exactly the same as those of glycollic acid. In addition, the physiological response of the sporangia at these concentrations is very nearly the same.

Summary of Table IV

Eight dicarboxylic acids of the aliphatic series were investigated for their toxic action upon germination of the sporangia of *Phytophthora colocasiae*. The results are summarized in Table IV.

Table IV.—*Toxic effect of di- and tri-carboxylic fatty acids on the spores of Phytophthora colocasiae Rac.*

Compound	Number of experiments	Number of cultures	Indirect germination			$H^+ + R^-$ [b]	HR [c]
			Critical concentration, normal	Lethal concentration, normal	a at 10° C. [a]		
Saturated dicarboxylic acids:							
Oxalic acid	3	46	0.0015		100	0.0015	0.0
Malonic acid	3	46	.003	0.004	42.48(4.9)	.00127	.00173
Succinic acid	3	44	.002		16 (5.7)	.00032	.00168
Unsaturated dicarboxylic acids:							
Maleic acid	4	40	.0015	.003	90.86(12)	.00136	.00014
Fumaric acid	3	36	.0009		65.26(12)	.000587	.000413
Hydroxydicarboxylic acids:							
Malic acid	3	34	.002				
Tartaric acid	4	42	.006	.007			
Hydroxytricarboxylic acid:							
Citric acid	3	38	.0025		38.91	.000972	.00153

TABLE IV.—*Toxic effect of di- and tri-carboxylic fatty acids on the spores of Phytophthora colocasiae Rac.*—Continued

Compound	Number of experiments	Number of cultures	Direct germination		α at 35° C.[a]	$H^+ + R^-$[b]	HR[c]
			Critical concentration, normal	Lethal concentration, normal			
Saturated dicarboxylic acids:							
Oxalic acid	3	46	0.002	0.003	100	0.002	0.0
Malonic acid	3	46	.004	.005	40.71	.00162	.00238
Succinic acid	3	44	.015	.02	6.05	.000907	.0141
Unsaturated dicarboxylic acids:							
Maleic acid	4	40	.003	.004	81.62	.00244	.000552
Fumaric acid	3	36	.004	-------	37.06	.00148	.00252
Hydroxydicarboxylic acids:							
Malic acid	3	34	.006	.007	-------	-------	-------
Tartaric acid	4	42	.006	.006	-------	-------	-------
Hydroxytricarboxylic acid:							
Citric acid	3	38	.008	-------	28.3	.00226	.00574

[a] The value of α is the percentage of an acid ionized at its critical concentration.
[b] $H^+ + R^-$ represents the concentration of the H-ions and the anions at the critical concentration.
[c] HR represents the concentration of the undissociated molecules at the critical concentration.
[d] The number in parentheses represents the temperature on the centigrade scale at which the value of α is determined.

Oxalic acid has about the same critical concentration for direct and indirect germination of the spores. In the case of direct germination, the toxicity gradient shows a sharp decline, for while in a 0.002 N solution of the acid there was slight germination, the sporangia were badly plasmolyzed in a 0.003 N solution. The ionization constant of the acid at 25° C. is approximately 63 times that of malonic acid, and 1,503.7 times that of succinic acid. Hence, we have another rule that "the nearer the substituent is to the carboxyl group, the greater is its effect on the ionization constant" (*14*). Moreover, this is also in accord with the general principle that "in a homologous series of organic acids the lower members of the series are the stronger" (*5, p. 87–144*). However, it should be remembered that the strength of an acid, expressed in terms of its ionization constant, does not necessarily imply a corresponding increase in its toxic action; for in the expression of the latter some other factors, such as the poisonous actions of the anions and the undissociated molecules, are involved. At its critical concentration the acid is practically completely dissociated. Hence, its toxic action at this dilution is due mainly to the effect of the H-ions. A study of the sodium salts showed that the anions do not appreciably influence the total toxicity of the acid.

For direct and indirect germination of the spores, malonic acid appears to exert about the same poisonous action. However, as in the case of oxalic acid, there appears an abrupt rise in the toxic value of the acid for direct germination, for while a 0.004 N solution of the acid allows germination of the sporangia, they show plasmolysis in 0.005 N concentration. The acid at the critical concentrations for direct and indirect germination of the spores is about 40.71 and 42.48 per cent ionized, respectively. Its toxicity to the spores is one-half that of oxalic acid, which exerts its toxic action mainly through the medium of the H-ions. From Table IV it will be seen that the toxic action of malonic acid also is due mostly to the H-ions

that the solution contains, although the effects of the anions and the undissociated portion on the toxicity have not been investigated.

Succinic acid shows a very peculiar behavior toward the two types of germination. While a 0.002 N solution of the acid inhibits the liberation of zoospores, the production of germ tubes is not prevented until a solution about seven times as concentrated as the one which just inhibits indirect germination is obtained. The remarkable specific toxic action of succinic acid in preventing the formation of zoospores is noteworthy. The experiment was repeated for a number of times at two different periods, and gave essentially similar results; fresh stock solutions of the acid were prepared each time. Considering indirect germination of the spores, the acid is a little more toxic than malonic acid, although the electrolytic dissociation of the latter at its critical concentration is about three times that of succinic acid. Evidently, then, the specific toxicity of succinic acid is due to the poisonous action of the anions or to the undissociated portion.

Of the unsaturated dicarboxylic acids, maleic and fumaric acids were investigated. It has already been noticed in the case of the carboxylic fatty acids that ordinary isomeric acids have very nearly the same electrolytic dissociation; but maleic and fumaric acids present a different case. There is a marked difference between the values of the ionization constant of the two acids, which appears to be the result of the position of two hydroxyl groups with respect to each other. The stereo-chemical influence is thus worthy of note.

According to Ostwald's (*15*) determination, the electrolytic dissociation of maleic and fumaric acids at the dilution 1,024 is 92.8 and 63.9 per cent, respectively. If the toxicity of the two acids were due to the effect of the H-ions in the solutions, one should expect maleic acid to be more toxic than fumaric. This, however, is not the case, since for indirect germination of the spores a 0.0015 N solution of maleic acid exerts the same toxic effect as does a 0.0009 N solution of fumaric acid. The greater toxicity of fumaric acid to the formation of zoospores may be due to the relatively greater poisonous action of the anions or the undissociated portion, or it may be due to the fact that the *trans* form is physiologically more active than the *cis* form. A study of the sodium salts will decide as to which of these two explanations is tenable. But when we consider the relative toxicity of the two acids to the production of germ tubes, the suggestion that the *trans* form may be more toxic than the *cis* form does not seem to hold. In this case maleic acid (*cis* form) is slightly more toxic than fumaric. Evidently, then, the explanation has to be sought in the specificity of the action of the acids toward the two types of germination.

It should be pointed out that from a practical chemical view point the variation between the critical concentrations of maleic and fumaric acids is well within the range of experimental error, considering that the organic compounds were not tested for purity or for alkali content of water distilled in glass apparatus. This would tend to show that the toxicity of the two acids is determined chiefly by the H-ions, and that the anions and the undissociated portions have a rather slight influence. Moreover, it should be noted that a highly dissociated acid will not have the same killing effect as an acid solution of the same P_H but of lower dissociation will have; for it is almost impossible to keep the acidity of a highly dissociated acid solution constant over a period of time. However, the variation in

the toxic values of the acids for a number of trials is so small that it seems somewhat doubtful whether the criticisms offered have any value.

Passing to the hydroxydicarboxylic acids, we come to malic and tartaric acids. Malic acid exerts a specific inhibitory influence on the formation of zoospores. A 0.002 N solution of the acid prevents the liberation of zoospores, while the production of germ tubes is retarded in 0.006 N solution. According to Ostwald's (*15*) determination, the electrolytic dissociation of the acid at the dilution 500, which is the critical concentration for indirect germination, is about 36 per cent. It hardly seems possible, therefore, that the toxic action is solely dependent upon the H-ions in the solution. At the critical concentration for direct germination the acid is much less ionized; hence the anions and the undissociated portion may have some effect in determining the total toxicity.

Tartaric acid is not a very strong acid, the concentration inhibiting direct and indirect germination of the spores being 0.006 N. At this concentration the acid is approximately 33 per cent ionized. The extent to which the anions and the undissociated portion influence the toxicity of the acid does not seem to be appreciably great.

Citric acid, a hydroxytricarboxylic acid, exerts a specific toxic action on the formation of zoospores. It inhibits the liberation of zoospores at 0.0025 N, while the production of germ tubes is prevented in 0.008 N solution. According to Jones's (*5, p. 87–144*) determination, the electrolytic dissociation of the acid at the critical concentrations for direct and indirect germination of the spores is approximately 28 and 38 per cent, respectively. The toxicity of the acid is due mostly to the effect of the H-ions in the solution.

Summary of Table V

Fifteen acids from the aromatic series were tested for their toxicity to the sporangia of *Phytophthora colocasiae*. By referring to Table V it will be seen that benzoic acid has a specific toxic action upon the liberation of zoospores. While a 0.0006 N solution of the acid prevents the formation of zoospores, it is only when a 0.002 N concentration is reached that the production of germ tubes is inhibited. In other words, the inhibitory effect of benzoic acid is about 3.3 times more effective in preventing indirect germination than in retarding the production of germ tubes. According to Jones's (*5, p. 87–144*) determination, benzoic acid at its critical concentrations for direct and indirect germination is about 16.69 and 27.39 per cent ionized, respectively; in other words, the difference between the electrolytic dissociation at the critical concentrations for direct and indirect germination is approximately 11 per cent. Moreover, it will be seen from Table VI the toxic values of the anions and the undissociated molecules in retarding the production of germ tubes are very nearly the same. However, inspection of Tables VI and VII will point out the fact that the value of the undissociated molecules in preventing the liberation of zoospores is more than four times the value calculated for direct germination. Also, the toxic value of the anions in inhibiting indirect germination is about three times that calculated for direct germination. Putting all these facts together, it will be easy of explanation as to how benzoic acid exerts a specific inhibitory action upon indirect germination of the spores.

TABLE V.—*Toxic effect of aromatic acids on the spores of Phytophthora colocasiae Rac.*

Compound	Number of experiments	Number of cultures	Indirect germination: Critical concentration, normal	Indirect germination: Lethal concentration, normal	α at 10° C.[a]	$H^+ + R^-$[b]	HR[c]
A. Monocarboxylic acid:							
I. Saturated—							
a. Benzoic acid	3	28	0.0006	------	[d]27.39(15.8)	0.000164	0.000434
b. Substituted acids—							
Hippuric acid	3	28	.002	0.003	28.36(12)	.000567	.00144
o-hydroxybenzoic acid	3	26	.0006	.0007	66.83(6.9)	.0004	.0002
m-hydroxybenzoic acid	3	26	.0009	------	25.58(13.22)	.00023	.000667
p-hydroxybenzoic acid	3	40	.001	.003	15.13(13.23)	.000151	.000849
Gallic acid	5	34	.003	------	10.19(6.5)	.000304	.00265
o-nitrobenzoic acid	5	46	.0009	.001	86.25(15)	.000776	.000124
m-nitrobenzoic acid	3	28	.0004	.0005	------	------	------
p-nitrobenzoic acid	3	30	.0006	.0007	53.73(12)	.000322	.000278
Dinitrobenzoic acid (3, 5)	2	24	.0006	.0007	74.61(12)	.000447	.000153
II. Unsaturated—							
Cinnamic acid	4	34	.0007	------	19.4(5.3)	.000135	.000565
B. Dicarboxylic acids:							
o-phthalic acid	3	28	.001	------	65.78(8.23)	.000657	.000343
C. Amino acids:							
Aspartic acid	6	64	.004	.005	------	------	------
D. Sulphonic acids:							
Sulphanilic acid	2	24	.002	.003	35.33(6.3)	.000706	.00129
E. Tannic acid:	4	46	[e].0005	------	------	------	------

Compound	Number of experiments	Number of cultures	Direct germination: Critical concentration, normal	Direct germination: Lethal concentration, normal	α at 35° C.[a]	$H^+ + R^-$[b]	HR[c]
A. Monocarboxylic acid:							
I. Saturated—							
a. Benzoic acid	3	28	0.002	0.0025	16.69	0.000333	0.00167
b. Substituted acids—							
Hippuric acid	3	28	.002	.003	28.55	.000571	.00143
o-hydroxybenzoic acid	3	26	.0007	.0008	67.31	.000471	.000229
m-hydroxybenzoic acid	3	26	.001	.002	24.44	.000244	.000756
p-hydroxybenzoic acid	3	40	.001	.002	15.50	.000155	.000845
Gallic acid	5	34	.007	------	7.08	.000495	.00651
o-nitrobenzoic acid	5	46	.0009	.001	84.5	.00076	.00014
m-nitrobenzoic acid	3	28	.0004	.0005	------	------	------
p-nitrobenzoic acid	3	30	.0006	.0007	54.38	.000326	.000274
Dinitrobenzoic acid (3,5)	2	24	.0006	.0007	75.75	.000454	.000146
II. Unsaturated—							
Cinnamic acid	4	34	.0007	.0008	20.08	.00014	.00056
B. Dicarboxylic acids:							
o-phthalic acid	3	28	.001	.002	64.09	.00064	.00036
C. Amino acids:							
Aspartic acid	6	64	.05	.06	------	------	------
D. Sulphonic acids:							
Sulphanilic acid	2	24	.003	.004	36.67	.0011	.0019
E. Tannic acid:	4	46	.006	------	------	------	------

[a] The value of α is the percentage of an acid ionized at its critical concentration.
[b] $H^+ + R^-$ represents the concentration of the H-ions and the anions at the critical concentration.
[c] HR represents the concentration of the undissociated molecules at the critical concentration.
[d] The number in parentheses represents the temperature on the centigrade scale at which the value of α is determined.
[e] Molar concentration.

TABLE VI.—*Relative toxic properties of the undissociated molecules as measured by their inhibiting power on the direct germination of the spores of Phytophthora colocasiae Rac.*

m-Nitrobenzoic acid	Salicylic acid	Hippuric acid	Benzoic acid	Hydrochloric acid	Agent
0.004 N	0.0007 N	0.002 N	0.002 N	0.002 N	Critical concentration.
500	286	100	100	100	Relative toxic value of HCl=100.
60	67.3	28	16	.....	Per cent ionized at critical concentration.
60	67.3	28	16	.....	Toxic value of cation in terms of ionic H^+.
1	1	.66	1	.....	Toxic value of anion in terms of ionic H^+.
61	68.3	28.66	17	.....	Total, cation + anion.
439	217.7	71.34	83	.....	Residual units.
40	32.7	72	84	.....	Per cent un-ionized.
10.97	6.65	.99	.98	.....	Value of un-ionized molecules in terms of ionic H^+.

TABLE VII.—*Relative toxic properties of the undissociated molecules as measured by their inhibiting power on the indirect germination of the spores of Phytophthora colocasiae Rac.*

m-Nitrobenzoic acid	Salicylic acid	Hippuric acid	Benzoic acid	Hydrochloric acid	Agent
0.0004	0.0006	0.002	0.0006N	0.002 N	Critical concentration.
500	333.3	100	333.3	100	Relative toxic value of HCl=100.
60	69.74	28	27.4	.....	Per cent ionized at critical concentration.
60	69.74	28	27.4	.....	Toxic value of cation in terms of ionic H^+.
2.2	6.6	2.2	3.2	.....	Toxic value of anion in terms of ionic H^+.
62.2	76.34	30.2	30.6	.....	Total, cation + anion.
437.8	256.96	69.8	302.7	.....	Residual units.
40	30.26	72	72.6	.....	Per cent ionized.
10.9	8.4	0.97	4.1	.....	Value of un-ionized molecules in terms of ionic H^+.

Hippuric acid, like benzoic acid, is relatively weakly dissociated. For direct and indirect germination the acid has the same critical concentration, viz, 0.002 N. Its electrolytic dissociation at this concentration is approximately 28 per cent, while at the same dilution benzoic acid is only about 16 per cent ionized but exerts the same toxic effect. It will be seen from Table VI that the relative toxic value of the anions of benzoic acid for direct germination is about one and one-half times that for hippuric acid, which, however, has the same toxic value of the undissociated molecules as benzoic acid. It therefore follows that the slight toxicity of benzoic acid due to low degree of electrolytic dissociation is so supplemented by the specific toxic action of the anions that the resultant of these factors gives a toxic value equal to that of hippuric acid, in the case of which the concentration of the H-ions make up any deficiency in toxicity due to relatively lower toxic value of the anions. With regard to indirect germination, the electrolytic dissociation of the two acids at their critical concentrations is approximately the same, although the relative toxic values of the anions and the undissociated portion of benzoic acid are greater than those of hippuric acid.

Turning to the substituted benzoic acids, we first come to the hydroxybenzoic acids. The three hydroxybenzoic acids differ greatly in regard to their toxicity to the spores. It will be seen from Table V that *o*-hydroxybenzoic acid (salicylic acid) prevents the liberation of zoospores in 0.0006 N solution, while *m*- and *p*-hydroxybenzoic acids exert the same effect at 0.0009 N and 0.001 N, respectively.

In the case of direct germination the order of toxicity is essentially the same, except that *m*- and *p*-hydroxybenzoic acids have the same critical concentration, viz, 0.001 N. The introduction of a hydroxyl group in benzoic acid and its position relative to the carboxyl group have a marked influence on the ionization constant of these acids. For instance, the substitution of the hydroxyl group in the position ortho to the carboxyl group in benzoic acid increases the constant of the acid 17 times, while the constant of *m*-hydroxybenzoic acid is only one and one-half times as great as that of benzoic acid. The case of *p*-hydroxybenzoic acid is quite peculiar; its dissociation constant, instead of increasing, decreases to one-half that of benzoic acid. The physiological action of the acids must be proportional to the degree of their electrolytic dissociation, if the toxicity of these acids is, to a large extent, determined by the effect of the H-ions. This is true, since the order of the toxicity of the acids is ortho, meta, and para. Since the sodium salts of the hydroxybenzoic acids, except sodium *o*-hydroxybenzoate, were not available, there was no means of comparing the relative toxic values of the anions and the undissociated portion. However, a glance at Tables VI and VII will show the relative toxic values of the anions and the undissociated molecules of *o*-hydroxybenzoic acid for direct and indirect germination.

Gallic acid, a trihydroxybenzoic acid, has an interest of its own. The introduction of three hydroxyl groups in benzoic acid, instead of increasing, decreases the constant of the acid to a little over one-half that of benzoic acid; but considering direct germination of the spores, the toxicity of gallic acid is one-third that of benzoic acid. The concentration of gallic acid which prevents the liberation of zoospores is 0.003 N, while the production of germ tubes is not inhibited until a 0.007 N concentration is reached, the latter concentration being twice the critical concentration for indirect germination. Evidently, then, gallic acid, like benzoic acid, exerts a specific toxic action on the formation of zoospores, the concentration of the acid preventing germination by the production of hyphae being twice that which exerts the same effect on indirect germination.

The other interesting group of the substituted benzoic acids is that of nitrobenzoic acids. By referring to Table V it will be seen that the order of the toxicity of these acids is not the same as that of the hydroxybenzoic acids. For the substitution of a nitro group in the position para to the hydroxyl group in benzoic acid increases the constant of the acid 100 times, while the constant of *m*-nitrobenzoic acid is 16 times that of benzoic acid, and that of *p*-nitrobenzoic acid about 6.6 times that of the latter. Thus we arrive at another general rule: "The relation between the dissociation constant of the para acid and that of ortho or meta acid varies with the nature of the substituent" (*14*). In other words, *p*-nitrobenzoic acid is about three times more ionized at the dilution 1,024 than *p*-hydroxybenzoic acid, for the nature of the nitro group is quite different from that of hydroxy. The critical concentrations of the three nitrobenzoic acids do not differ in regard to direct and indirect germination; that is, for each acid the concentration at which the liberation of zoospores is inhibited is the same as that which prevents the production of germ tubes. Considering indirect germination of the spores, *o*-nitrobenzoic acid retards germination in 0.0009 N solution, while *m*- and

p-nitrobenzoic acids exert the same effect at 0.0004 N and 0.0006 N, respectively. Since the order of the poisonous action of these acids does not correspond with the degree of their electrolytic dissociation, it follows that the effect of the H-ions alone is not responsible for the total toxicity, but the anions and the undissociated portion have a toxic action of their own. It was not possible to determine the extent to which the anions and the undissociated portion contribute to the toxicity of their respective acids, since the sodium salts of the two nitrobenzoic acids were not available for experimentation. Tables VI and VII will, however, show the relative toxic values of the anions and the undissociated portion of *m*-nitrobenzoic acid. It will be seen that the relative toxic value of the undissociated molecules of this acid is the greatest as compared with those of other acids in the table.

For direct and indirect germination of the spores, the critical concentration of 1-, 3-, 5-dinitrobenzoic acid is 0.0006 N. At this concentration the acid is about 75 per cent ionized, according to Jones's (*5, p. 87–144*) determination (by interpolation). Evidently the toxic action is determined chiefly by the effect of the H-ions at this dilution. Since the sodium salt of the acid was not available, no data as to the extent to which the anions and the undissociated molecules influence its toxicity are at hand.

Cinnamic acid is the best-known unsaturated acid of the aromatic series. At a concentration of 0.0007 N of the acid, there is inhibition of direct and indirect germination of the spores. According to Jones's (*5, p. 87–144*) determination, the electrolytic dissociation of the acid at its critical concentration is approximately 20 per cent. Hence the toxic effect of the H-ions is, indeed, not very great, since only one-fifth of the acid is ionized at these concentrations. Obviously, then, an explanation of the toxic action of the acid has to be sought in the poisonous action of the anions and the undissociated portion. It is also possible that the presence of an ethylenic linkage and a conjugated system in the acid may have to do materially in increasing its toxic action.

Phthalic acid is a dicarboxylic acid, and, according to Jones (*5, p. 87–144*), is about 65 per cent ionized at its critical concentrations. The introduction of a second carboxyl group has increased the strength of the acid, for its dissociation constant is 18.33 times that of benzoic acid. However, an increase in the degree of electrolytic dissociation of the acid has not enhanced its toxic action, since it inhibits germination of the spores at 0.001 N solution.

Aspartic acid is a monamino-dicarboxylic acid. The dissociation of the acid is not given in Ostwald's (*15*) or Jones's (*5, p. 87–144*) tables. The acid inhibits the liberation of zoospores in 0.004 N solution, while the production of germ tubes is not retarded until a 0.05 N concentration is reached; in other words, like benzoic acid, this acid shows a specific inhibitory action to the formation of zoospores. It will be of interest to know the extent to which the anions and the undissociated molecules are responsible for the toxicity of the acid. The electrolytic dissociation of the acid at its critical concentrations is certainly not very great.

For direct and indirect germination of the spores, sulphanilic acid has critical concentrations at 0.002 N and 0.003 N, respectively. According to Jones's (*5, p. 87–144*) determination, the electrolytic dissociation of the acid at its critical concentrations is approximately

36 per cent. Whether the poisonous action at these concentrations is due entirely to the effect of the H-ions or partly to that of the anions and the undissociated molecules could not be determined, as the sodium salt of the acid was not available for experimentation. Sulphanilic acid, however, is not a very strong acid, for the presence of an amino group lowers its strength.

For indirect germination of the spores, tannic acid has a critical concentration at 0.0005 M, while the production of germ tubes is not retarded until 0.006 M concentration of the acid is reached. Thus tannic acid exerts a specific inhibitory action against the formation of zoospores.

DISCUSSION

ALCOHOLS

Very few cases are known in which the physiological action of organic compounds can be predicted from a knowledge of their chemical constitution. This is probably due to the complex nature of many such substances, which makes any prediction as to their physiological action a rather difficult matter. Instances are known, however, of simple substances whose physiological effects can be foretold by their chemical and physical properties. A case of this kind is furnished by the so-called Richardson's law, which states that the toxicity of the normal aliphatic alcohols increases in proportion to their molecular weight.

Kamm (7) has recently brought forward facts to show that the physiological effects of the members of the normal aliphatic alcohols also admit of mathematical interpretation, which is a real contribution to our knowledge of the physiology of these compounds. For instance, he has found that in a homologous series of aliphatic alcohols the toxicity of a member was three times that of the preceding one. On this basis he has developed a mathematical relationship for determining the toxicity of any member in the series, having been given the toxicity of ethyl alcohol. Using his formula and expressing the toxicity of ethyl alcohol as 6.3, as found for the sporangia of *Phytophthora colocasiae*, the following figures are obtained for normal propyl and normal butyl alcohols:

(*a*) For normal propyl alcohol:

$$1 \times 3 = 3$$

$$\frac{6.3 \times 1.3}{3} = 2.73 \text{ (uncorrected for specific gravity).}$$

(*b*) For normal butyl alcohol:

$$1 \times 3 \times 3 = 9$$

$$\frac{6.3 \times 1.6}{9} = 1.12 \text{ (uncorrected for specific gravity).}$$

By reference to Table I, it will be seen that there is a very close correspondence between the values calculated by the above method and those experimentally determined for *Phytophthora colocasiae*. For instance, normal propyl alcohol was found to prevent the formation of zoospores in a 3.1 per cent solution, which compares

favorably with the calculated value of 2.73. Normal butyl alcohol has experimental and calculated values of toxicity of 1.5 and 1.2 per cent, respectively. However, it should be remembered that the calculated values are not corrected for the specific gravity of the corresponding alcohols, which may explain the slight difference between the experimental and the calculated values.

It has been found, as shown by various investigators, that for bacteria and mammals the toxicity of the normal fatty alcohols increases as the homologous series is ascended; that is, the alcohols having higher boiling point, specific gravity, and heat of combustion, and lower solubility have the greater toxic value. In other words the phenomenon of toxicity is a function of the chemical and physical properties of these substances.

According to the writer's results, methyl alcohol has not proved an exception to the general rule that the toxicity of the normal aliphatic alcohols increases with molecular weight. These results are in agreement with the findings of Vernon (*20*), Macht (*11*), and Kuno (*8*), in the case of mammals; and Stadler (*18*), in the case of bacteria. These results lead the writer to conlude with Vandevelde (*19*) and Förster (*4*) that any failure of methyl alcohol to behave in accordance with the rule of increasing molecular weight is due to impurities in the alcohol. Moreover, Macht (*11*) has pointed out that the toxic effects of methyl alcohol, when taken by mouth into the alimentary canal, are due to its decomposition products, especially formic acid and formaldehyde. However, such conditions are not obtained in a drop of methyl alcohol in which fungous or bacterial spores are sown, for methyl alcohol, under these conditions, does not change into formic acid or formaldehyde, with the result that the toxicity of the drop is not altered. Therefore, methyl alcohol as such is not more toxic to the spores than ethyl alcohol.

The isomeric alcohols were found to be less toxic than the corresponding normal alcohols, thus showing that a change in the structural constitution of an alcohol was accompanied by a change in its toxicity, although the molecular weight remained the same. In the case of butyl alcohols, Overton (*16, p. 101,*) has shown that of isomeric alcohols those having the least-branched carbon chain have the strongest narcotic action, while those with the most-branched carbon chain have the weakest action. However, Vernon (*20*) found, in the case of the depressant action of alcohols on the heart beats of the frog, that the behavior of secondary butyl alcohol was contrary to the rule laid down by Overton for the toxicity of isomeric butyl alcohols. The writer's results are in agreement with those of Vernon, and show that Overton's rule does not hold good for indirect germination of the sporangia of *Phytophthora colocasiae*.

The high toxic value of allyl alcohol may be due to the allyl radical that it contains. The presence of a double bond has also, no doubt, an accelerating influence on the toxicity of the alcohol. Glycerol and ethylene glycol, being the lowest members of the homologous series, are not very toxic. The toxicity of benzyl alcohol is probably determined by the benzene nucleus, while that of furfuralcohol may be due to the unsaturated oxygen atom in the furyl radical.

The close approximation of the results obtained in these investigations with those of other workers emphasizes the important fact that the relative physiological action of alcohols is the same for living

matter—amphibians, mammals, higher plants, bacteria, and fungi. However, it is very doubtful whether alcohols have any desirable features as fungicides, for their high cost and comparatively low toxicity will prohibit their use in any commercial work. Allyl alcohol, however, is about as toxic to the spores as copper sulphate, but it is many times more expensive than the latter. Its strong, irritant odor is also a disagreeable feature.

ALDEHYDES

The results of tests on the toxicity of aldehydes show that the toxic properties of aldehydes are due mostly to the aldehyde group which they contain, and that the aromatic or the aliphatic nature of a radical, to which the aldehyde group is united, does not seem to influence the toxicity of the compounds to any marked degree. For example, normal butyraldehyde which contains a propyl (C_3H_7) radical directly united to an aldehyde group, in place of a furyl (C_4H_3O) radical as in furfuraldehyde, shows about the same toxicity as the latter. Indeed, it was surprising to find that the unsaturated oxygen atom in the closed chain of furfural did not have any accelerating effect on the toxicity of the compound. Therefore, it is safe to assume that the furyl and the alkyl radicals are equally toxic, if at all. However, these radicals are so different in their chemical properties that their physiological action would hardly be similar, with the result that the aldehydes should show different toxic values. This is, however, contrary to the experimental data, which show that all aldehydes containing one aldehyde group, except the substituted benzaldehydes and formaldehyde, were equally toxic to the spores. It follows, therefore, that the toxic action due to the alkyl radicals must be negligible. It has also been shown that the toxicity of the aliphatic aldehydes, unlike that of the aliphatic alcohols, does not increase with molecular weight.

The seemingly erratic results with formaldehyde need not surprise us at all, for it corresponds with anomalies observed in regard to its chemical and physical properties. For instance, it is known that formic acid, the first member of the fatty acid series, differs markedly in some of its reactions from acetic acid and other members of the series; the same relations have been observed in the first members of other homologous series. Hence, the reactions of formaldehyde are not as representative of the group as are those of acetaldehyde. It is therefore not surprising to find that its toxicity to the spores is different from that of other aldehydes in the series. Moreover, it is also possible that the presence of a hydrogen atom in formaldehyde, which is very sensitive to oxygen, may have an accelerating influence on its toxicity.

The results of tests on the toxicity of glyoxal are very instructive. Glyoxal contains two aldehyde groups without having any alkyl radical united to them. Evidently, then, the toxicity of this compound must be an expression of the physiological action of the aldehyde groups, which is not influenced by any other group or radical in the molecule. Considering that the poisonous action of glyoxal is an expression of the toxic value of its two aldehyde groups, it may be expected that the toxicity of such aldehydes as contain only one aldehyde group must be about one-half that of glyoxal.

However, glyoxal is about 10 times as toxic as any of the aliphatic aldehydes containing only one aldehyde group, excepting formaldehyde. This would seem to indicate that the presence of an alkyl radical, in fact any radical, in aldehydes, instead of increasing, diminishes their physiological action.

Moore (*12*) generalized that "up to 250° C. the higher the boiling point the more toxic the compound to insects." This, however, does not apply to germination of the sporangia of *Phytophthora colocasiae*. For glyoxal, which has a boiling point (50°), is the most toxic of the aldehydes used in these experiments, excepting formaldehyde and the substituted benzaldehydes, whereas benzaldehyde with a boiling point of 179.5° is much less toxic than glyoxal. Moreover, Moore thought that the lessened toxicity of furfural was due to its low boiling point, 90°. The furfural used by the writer in these experiments had a boiling point of 160°. However, acetaldehyde, which has a boiling point about one-sixth as high as that of furfural, showed much the same toxicity to the spores as did the latter.

The substituted benzaldehydes show a decided increase in toxicity over that of benzaldehyde. The introduction of the nitro and the hydroxyl groups into the benzene nucleus of benzaldehyde increases the toxicity of the compound to a marked degree. In the two nitrobenzaldehydes investigated, the position of the substituent with respect to the aldehyde group does not make any difference in their physiological action. However, the substitution of a hydroxyl group in the position para to the aldehyde group in benzaldehyde increases the toxicity of the compound over that of *p*-nitrobenzaldehyde. The substitution of two hydroxyl groups does not result in any further increase in toxicity, because protocatechuic aldehyde and *p*-hydroxybenzaldehyde are about equally toxic. Whether the increase in toxicity of the substituted benzaldehydes is due to the position of the substituent with respect to the aldehyde group or to the nature of the substituent, could not be definitely determined, since *o*- and *m*-hydroxybenzaldehydes did not lend themselves to experimentation, being very slightly soluble in water. However, it will be seen from Table II that protocatechuic aldehyde and *p*-hydroxybenzaldehyde are more toxic than *m*- or *o*-nitrobenzaldehyde. It seems, therefore, that the substitution of a hydroxyl group in benzaldehyde increases its toxicity more than does that of a nitro group.

ACIDS

The results on the toxicity of organic acids to the sporangia of *Phytophthora colocasiae* are not in agreement with those of Kahlenberg and True (*6*), who investigated the toxicity of a number of organic and inorganic compounds to the seedlings of *Lupinus albus* L. All the acids used in these present experiments were found to be toxic to the spores at concentrations higher than those which were found by Kahlenberg and True to kill the lupines. However, it should be noted that, with some exceptions, the order of the toxicity of most of the acids investigated corresponds with that determined for the seedlings of *L. albus* L. If the acids used by Kahlenberg and True were pure chemically, the differential behavior of the compounds is of great significance in their use as fungicides when applied on the host.

It should be pointed out that the organic compounds used in this research were not tested for purity in the laboratory, and all conclusions are based on the assumption that they were pure chemically. Every effort was made to obtain chemicals of the highest purity, yet they could not be compared for purity with chemicals prepared in the laboratory for experimentation. However, in biological work, the variation in results obtained with compounds of "C. P." purity and of "absolute" purity is so small that the general conclusions of an experiment would not be affected appreciably.

SUMMARY

The results on the toxicity of the aliphatic alcohols substantiate Richardson's law, which states that the toxicity of the normal aliphatic alcohols increases with molecular weight.

The isomeric aliphatic alcohols were found to be less toxic than the corresponding normal alcohols. Normal butyl, isobutyl, secondary butyl, and tertiary butyl alcohols were decreasingly toxic in the order mentioned.

Allyl alcohol was the most toxic of the alcohols investigated, being 12,600 times more toxic than ethyl alcohol, which was toxic at a concentration of 6.3 per cent. The high toxic value of allyl alcohol is due probably to the allyl radical. The presence of a double bond has also an accelerating influence on its toxicity.

Ethylene glycol and glycerol were not very toxic, being the lowest members of the homologous series.

The toxicity of benzyl alcohol and furfuralcohol was 31 times greater than that of ethyl alcohol. The toxicity of benzyl alcohol is due probably to the benzene nucleus, while that of furfuralcohol may be due to the presence of the unsaturated oxygen atom in the furyl radical.

The toxicity of aldehydes is due mostly to the aldehyde group which they contain, and the aromatic or the aliphatic nature of a radical to which the aldehyde group is united seems to have very little influence on their toxicity.

Unlike the normal aliphatic alcohols, the toxicity of aldehydes did not increase as the homologous series was ascended.

The increased toxicity of formaldehyde corresponded with anomalies observed in regard to its chemical and physical properties. It is possible that the presence of a hydrogen atom in formaldehyde which is very sensitive to oxygen may have an accelerating influence on its toxicity.

The toxicity of glyoxal is an expression of the toxic value of the two aldehyde groups which it contains. It was much more toxic than any other aldehyde investigated, excepting formaldehyde and the substituted benzaldehydes.

The substitution of a nitro or a hydroxyl group in benzaldehyde increased its toxicity many times. The substitution of two hydroxyl groups did not result in any further increase in toxicity. The hydroxybenzaldehydes were more toxic than the nitrobenzaldehydes.

The results on the toxicity of organic acids signify that the electrolytic dissociation of an organic acid is not always an index of its physiological action, for in these acids the undissociated molecules and the anions had a distinct toxic action of their own.

Disregarding the toxic action of the undissociated portion and the anions, in a homologous series of organic acids the lower members of the series were stronger. In general, the ordinary isomeric acids had very nearly the same toxicity.

The introduction of a halogen into acetic acid enormously increased its dissociation, and consequently its physiological action, though not to the same extent. The three chloro-acetic acids were found to be equally toxic. The high toxic value of monobromacetic acid is probably due to the action of the anions.

The introduction of a hydroxyl group into acetic and propionic acids increased their electrical conductivity, with a consequent increase in their toxic action.

"The nearer the substituent is to the carboxyl group, the greater is its effect on ionization constant." However, this was not true with respect to the physiological action of oxalic, malonic, and succinic acids, although their electrolytic dissociation is governed in accordance with the above formula.

The stereoisomerism, as illustrated by maleic and fumaric acids, had a marked influence on the physiological action of these acids. Fumaric acid (*trans* form), which has a lower dissociation than maleic acid (*cis* form), was more toxic than the latter.

The nature of a substituent in an aromatic acid and its position relative to the carboxyl group generally determine the toxic action of the acid. The nitrobenzoic acids differed in toxicity from the hydroxybenzoic. The order of the toxicity of the hydroxybenzoic acids was ortho, meta, and para, whereas for the nitrobenzoic acids it was meta, para, and ortho.

A hydroxyl or a nitro group in benzoic acid increased the dissociation constant of the acid, with a consequent increase in toxicity. However, in the case of indirect germination, an increase in the dissociation constant of the acid was not always accompanied by an increase in its toxicity. The introduction of three hydroxyl groups in benzoic acid, instead of increasing, decreased its dissociation constant, and consequently its toxicity.

A double bond led to an increased physiological activity of the acid, as in cinnamic acid. It is possible that a conjugated system also accelerated its toxicity.

The introduction of a second carboxyl group into benzoic acid increased its dissociation, but not its toxicity, as noted with *o*-phthalic acid.

The presence of an amino group lowered the toxicity of sulphanilic acid.

Benzoic, succinic, and tannic acids showed specific inhibitory action against the formation of zoospores in the sporangia of *Phytophthora colocasiae* Rac.

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